농학석사 학위논문

Pathobiome revealed by transgenic rice lines with altered immunity and rice with *Magnaporthe oryzae* infection

면역 유전자 변이 품종과 벼도열병 감염으로 밝혀낸 벼의 패쏘바이옴

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By

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Department of Agricultural Biotechnology

The Graduate School of Seoul National University

August 2023

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지도교수 이 용 환

이 논문을 농학석사 학위논문으로 제출함

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Pathobiome revealed by Transgenic rice lines with altered immunity and rice with *Magnaporthe oryzae* infection

UNDER THE DIRECTION OF DR. YONG-HWAN LEE

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY
BY
TAEON CHRISTOPHER KIM

PROGRAM IN PLANT MICROBIOLOGY
DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY

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ABSTRACT

Pathobiome revealed by transgenic rice lines with altered immunity and rice with *Magnaporthe oryzae* infection

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Pathobiome is a burgeoning field in microbiome research that focuses on studying diseases at a microbiome scale. It describes the biotic environment interacting with a pathogen or dysbiosis, thus providing valuable insights into disease dynamics. This study investigates the pathobiome of rice blast, a critical disease that accounts for up to 30% of damage in rice production. Two experiments were conducted to examine the rice pathobiome. The first experiment explored the pathobiome of the leaf endosphere of transgenic rice plants with altered immunity against rice blast. The first experiment compared Nakdong and its transgenic variety *MoHTR1_OX,* as well as Dongjin and its transgenic variety *OsMORE1a*. The second experiment identified the pathobiome of the infected leaf endosphere during rice blast infection. The comparison was between rice infected and uninfected with

Magnaporthe oryzae on two rice cultivars, Nakdong and Dongjin. Results revealed that the alpha diversity of Observed ASV, Shannon index, and Simpson index did not show significant differences in both the genetically altered immunity experiments comparing transgenic plants with wildtype plants and infection experiments comparing infected plants with uninfected plants. However, beta diversity significantly differed between transgenic and wildtype plants, demonstrating clear separation. Similarly, infected and uninfected plants were clearly distinguishable. Furthermore, individual genera and ASVs exhibited differential enrichment based on community composition and differential abundance analyses. These findings hold potential for future studies concerning pathobiome endophytes, which respond to pathogen infection and plant genetic manipulation, thereby providing an understanding of the tripartite relationship between rice, *M. oryzae*, and the endophytes.

Keywords: Pathobiome, *Magnaporthe oryzae*, transgenic, altered immunity, rice

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INTRODUCTION

Rice is a staple crop for at least three billion people (Skamnioti and Gurr, 2009). Its importance makes rice health a vital subject for stable rice production. Rice health depends on various factors, including the microbiome, which contributes to growth enhancement and disease resistance (Busby et al., 2017; Song et al., 2020; Zhang et al., 2021). A balanced microbiome strives for homeostasis against intruding microorganisms like pathogens (Paasch and He, 2021), where plant immunity plays a crucial role (Dastogeer et al., 2020).

However, dysbiosis caused by pathogen introduction, environmental factors, or genetic defects can lead to the microbiome transforming into a pathobiome associated with reduced host immunity (Defazio et al., 2014; Krezalek et al., 2016). In rice, one major cause of a pathobiome is the fungal pathogen *Magnaporthe oryzae,* responsible for rice blast, which can decrease rice production by up to 30%, an equivalent to the worth of food for 60 million people (Skamnioti and Gurr, 2009; Kirtphaiboon et al., 2021). Rice blast symptoms significantly impact the rice microbiome. Additionally, an alteration of plant immunity due to genetic changes is another cause of the rice pathobiome. Several attempts from other studies have been made to modify rice immunity against *M. oryzae*.

The effect of altered immunity due to genetic modification or pathogen introduction impacts individual microorganisms, leading toward a pathobiome. There are three modes of action for a phytopathogen to infect its host, which are

necrotrophic, biotrophic, and hemibiotrophic. A necrotrophic pathogen kills the host for nutrient acquisition (Laluk and Mengiste, 2010). A biotrophic pathogen keeps the host alive to extract nutrients as it suppresses the plant immune system, while a hemibiotrophic pathogen, like *M. oryzae*, is biotrophic first and then switches to necrotrophic (Perfect and Green, 2001).

One example of a genetic change in rice immunity related to the three modes of pathogenicity is the introduction of the MoHTR1 (*Magnaporthe oryzae* Host Transcription Reprogramming 1) nuclear effector from *M. oryzae* to the rice genome (Kim et al., 2020). MoHTR1 represses the transcription of many immunity genes in rice along with 19 other *MoHTR* genes. MoHTR1 lowers plant immunity towards *Xanthomonas oryzae* pv. *oryzae*, a hemibiotrophic bacterial pathogen, but increases resistance in *Cochliobolus miyabeanus*, a necrotrophic fungal pathogen when incorporated into the rice genome (Kim et al., 2020). Meanwhile, a rice gene, *OsMORE1a* (*Oryza sativa Magnaporthe oryzae* Resistance 1a), shows a comparable immunity effect regarding hemibiotrophic and necrotrophic pathogens (Kim et al., 2022).

OsMORE1a is one of the 13 *OsMORE1* genes in rice that are homologs of the *MORE1* gene in *Arabidopsis thaliana*, responsible for GH10 (glycoside hydrolase 10) synthesis (Kim et al., 2022). GH10 primarily involves cell wall expansion as a xylanase but also serves roles in plant immunity (Lu et al., 2020). The mutation of the *OsMORE1a* gene increased resistance against *M. oryzae* fungal infection in rice, resulting in a far fewer lesion numbers and sizes (Kim et al., 2022). Moreover, the *OsMORE1a* mutation in rice also increased rice resistance towards *X.*

oryzae pv. *oryzae*, a hemibiotrophic bacterial pathogen, but enhanced susceptibility towards *C. miyabeanus*, a necrotrophic pathogen. Consequently, the effects of *OsMORE1a* mutation and MoHTR1 overexpression showed opposites when confronted with these hemibiotrophic and necrotrophic pathogens.

Changes in microbial abundance between different genetic treatments are indicators of a pathobiome. One of the earliest mentions of the word "pathobiome" hinted at it being a seemingly normal microbiome containing pathogens becoming pathogenic under certain host conditions (Ryan, 2013). The definition of pathobiome evolved from a group of pathogens (Ryan, 2013; Defazio et al., 2014) to the microbial community shifting in abundance in the presence of a pathogen and dysbiosis (Vayssier-Taussat et al., 2014; Jakuschkin et al., 2016; Sweet et al., 2017; Bass et al., 2019; Doonan et al., 2019; Mannaa and Seo, 2021; Qiu et al., 2022). A study on transgenic rice with the R gene *Piz-t* and *M. oryzae* infection investigated the rice rhizosphere and root endosphere for hints of the pathobiome (Tian et al., 2021). However, no studies combined transgenic rice and *M. oryzae* infection investigation on the rice leaf endosphere in search of a pathobiome.

Leaf endophytes are among the least studied plant microbiomes due to the overwhelming presence of mitochondria and chloroplast 16S rRNA data, which is now less of a problem due to PNA clamps that inhibit mitochondria and chloroplast sequences (Lundberg et al., 2013). Endophytes are microbial communities that reside in a plant endosphere without disease symptoms since they can modulate plant immunity (Compant et al., 2010; Kandel et al., 2017). Therefore, changes in plant immunity due to dysbiosis caused by pathogen introduction and genetic

modification have considerable impacts on the endophytes. This study aims to reveal the composition shifts of leaf endophytes under dysbiosis conditions. Two experiments for this study evaluated leaf endophytes of *M. oryzae*-infected leaves and genetically altered leaves to compare their community changes.

MATERIALS AND METHODS

I. Sample sets

Four different rice varieties were used for this study, which are Nakdong and *MoHTR1_OX,* provided by Kim et al. (2020), and Dongjin and *OsMORE1a,* provided by Kim et al. (2022). *MoHTR1_OX* is a transgenic Nakdong variety that overexpresses the MoHTR1 (*M. oryzae* Host Transcription Reprogramming 1) nuclear effector of *M. oryzae* KJ 201, which alters plant immunity. *OsMORE1a* is a transgenic Dongjin variety that had the *OsMORE1a* mutation. The four sets of this research are, samples comparing bacteria and fungi of Nakdong and *MoHTR1_OX,* samples comparing bacteria and fungi of Dongjin and *OsMORE1a*, samples comparing bacteria of *M. oryzae* KJ 201 infected and uninfected Nakdong (48 hpi and 72 hpi), and samples comparing bacteria of *M. oryzae* PO 6-6 infected and uninfected Dongjin (48 hpi and 72 hpi). Each set had three replicates.

II. Rice sample preparation

Seeds were surface-sterilized in 70% ethanol for 2 minutes, 2% NaOCl for 2 minutes, 70% ethanol for 1 minute, and washed with sterilized deionized water 10 times. Subsequently, seeds were stored at 4°C until further use. Next, pots filled with potting soil from Punong Co., Ltd were autoclaved at 121°C for 20 minutes, with aluminum foil covering the top to prevent contamination. The surface-sterilized seeds were sprouted in Petri dishes with autoclaved mesh moistened with

autoclaved deionized water for 4 days. After sprouting, the seeds were transferred to pots placed in growth chambers at 25°C under 80% humidity. Each pot contained four replicates of seeds, and the plants were grown for 4 weeks. Throughout the growth period, the plants were watered only with autoclaved deionized water. For infection assays, rice plants were infected with *M. oryzae* spores grown in V8 agar (8% V8 juice and 310 ppm 10N NaOH) for 7 days, with a concentration of 5 X 10^5 spores per mL in 250 ppm Tween 20. The infected plants were then placed in dark chambers with 100% humidity for 24 hours. In contrast, rice plants designated as uninfected were sprayed with 250 ppm Tween 20 without *M. oryzae* spores. After the infection process, the third leaves from the bottom of each plant were harvested and subjected to surface-sterilization, following the same procedure as earlier (70% ethanol for 2 minutes, 2% NaOCl for 2 minutes, 70% ethanol for 1 minute, and washed with sterilized deionized water 10 times). The cleaned leaf samples were then stored at -75 °C until DNA extraction.

III. DNA extraction and amplification

Each sample was transferred to a Lysing Matrix S tube (MP Biomedicals) and ground using the FastPrep-24™ 5G homogenizer (MP Biomedicals). DNA was extracted using the SPINeasy DNA Kit for Soil (MP Biomedicals). DNA concentration was measured using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific) and adjusted to 40 ng/uL for each PCR reaction tube. Each sample had three technical replicates for PCR. The primers contained Illumina adaptors for

Mi-seq analysis (Bionics) (Table 1.). Bacterial primers were 515F-Mi and 806R-Mi, amplifying the V4 region of the 16S ribosomal RNA gene (Caporaso et al., 2011). Fungal primers were ITS1F-Mi and ITS2R-Mi, amplifying the ITS1 region of the 18S ribosomal RNA gene (Op De Beeck et al., 2014). During bacterial DNA amplification, mitochondrial and plastid (chloroplast) DNA were hindered from amplification using PNA (peptide nucleic acid) clamps (Panagene) (Lundberg et al., 2013) (Table 1.). The PNAs used were mPNA for mitochondria and pPNA for plastid. The PCR mixture used for this study was Invitrogen 2X Platinum SuperFi™ II Green PCR Master Mix (Thermo Fisher Scientific). For the bacterial PCR, a 25 uL PCR mix included 12.5 uL of 0.5 uM master mix, 1 uL of 0.5 uM forward primer, 1 uL of 0.5 uM reverse primer, 1.5 uL of 0.75 uM mPNA, 1.5 uL of 0.75 uM pPNA, and 7.5 uL of DNA with deionized water. The 25 uL PCR mix for fungi was the same but without mPNA and pPNA. The PCR steps were as follows: initial denaturation at 98°C for 30 seconds, then repeating 32 cycles of denaturation at 98°C for 10 seconds, PNA annealing at 78°C for 10 seconds (only for bacteria), primer annealing at 55°C for 10 seconds, extension at 72°C for 1 minute 40 seconds, then back to denaturing for the 32 cycles, final extension at 72°C for 5 minutes, and holding at 12°C. Successful samples were purified with MEGAquick-spin™ Plus Fragment DNA Purification Kit (Intron Biotechnology). The samples were then sent to NICEM (National Instrumental Center for Environmental Management) at Seoul National University, Korea to undergo the second round of PCR with the Nextera XT Index Kit (Illumina). DNA concentrations were equally adjusted with Infinite

200 pro (Tecan). Library pooling and concentration were done with AMPure beads (Beckman Coulter). Gel purification selected PCR products with a 2 × 300 bp read length on the Illumina Mi-Seq platform.

IV. DNA sequencing

The QIIME2 pipeline (v. 2022.8) was used to process DNA sequences (Bolyen et al., 2019). Sequences were demultiplexed, merged, and quality filtered using the DADA2 plugin (Callahan et al., 2016), and labeled as amplicon sequence variants (ASVs). For bacteria, non-chimeric ASV taxonomy assignment was performed using the Naïve Bayes algorithm of q2-feature-classifier (Bokulich et al., 2018) based on the Silva database (v.138) (Quast et al., 2012). For fungi, taxonomic assignment was done using the q2-feature-classifier based on the UNITE database (UNITE_ver8_dynamic of May 2021) (Nilsson et al., 2019). Subsequent statistical processing and analysis were conducted in R (v. 4.2.3) (R Core Team, 2023) with a default significance level of $\alpha = 0.05$. The R package phyloseq (v. 1.40) (McMurdie and Holmes, 2013) was used to import ASV tables for R coding. Chloroplast and Rickettsiales ASVs were removed from bacterial data, while Chromista, Plantae, and unassigned ASVs were removed from fungal data. Data visualization was performed using the R package ggplot2 (v. 3.4.2) (Wickham, 2016). To normalize ASV data, the R package metagenomeSeq (v. 1.38) (Paulson et al., 2013) was employed, utilizing cumulative-sum scaling (CSS) and log-transformation with the cumNorm() and MRcounts() functions.

V. Microbial diversity and abundance visualization

Alpha diversity was determined using ASV rarefied with the rarefy_even_depth() function from the phyloseq package. The diversity() function from the vegan package (v.2.6-4) (Oksanen et al., 2022) was employed to calculate the Observed ASV, Shannon index, and Simpson index. For beta diversity analysis, the ordinate() function from the phyloseq package was used to construct CAP (Canonical analysis of principal coordinates) plots. ASVs with a relative abundance lower than 0.5% were classified as low abundance for community composition analysis. Differential abundance analysis was performed using the function fitZig() from metagenomeSeq, conducting a zero-inflated Gaussian distribution mixture model. Contrasting samples were labeled 0 and 1, and 66% of the total samples were randomly selected as training sets. The randomForest package (v.4.7.1.1) (Liaw and Wiener, 2002) was used as a classification method, and the top 20 ASVs were selected based on the mean decrease in the Gini coefficient. For co-occurrence network analysis, the sparcc() function with the SpiecEasi package (v. 1.1.2) (Kurtz et al., 2023) was used to perform SparCC analysis on CSS-normalized ASV data. Compositionality-robust correlations were measured with 1000 bootstrap samples for pseudo p-value inference using the the sparccboot() function from the SpiecEasi package. ASV associations were limited to correlations of > 0.3 and < -0.3 (p-value < 0.05). Additionally, ASV correlations were observed with degree(), betweenness(), and closeness() using the igraph package (version 1.4.2) (Csardi and Nepusz, 2006).

Table 1. Primers used in this study

Primer name	Sequence (5'→3')
For primers containing Illumina adaptors for Mi-seq analysis:	
515F-Mi	TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG CCA GCM GCC GCG GTA A
806R-Mi	GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACH VGG GTW TCT AAT
ITS1F-Mi	TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CTT GGT CAT TTA GAG GAA GTA A
ITS2R-Mi	GTC TCG TGG GCT CGG AGA TCG GTA TAA GAG ACA GGC TGC GTT CTT CAT CGA TGC
For PNA clamps:	
mPNA	GGC AAG TGT TCT TCG GA
pPNA	GGC TCA ACC CTG GAC AG

RESULTS

I. Nakdong and Dongjin had different endophytes

Nakdong and Dongjin, the two rice varieties used for this study, were compared based on their leaf endophyte communities. Alpha diversity did not differ significantly between endophytes of Nakdong and Dongjin leaves for both bacteria (Fig 1a) and fungi (Fig 1b). The observed alpha diversity indices were Observed ASV, Simpson Evenness, and Shannon Diversity. Regarding beta diversity, CAP (Canonical analysis of principal coordinates) showed the bacterial communities (Fig 2a) and fungal communities (Fig 2b) clearly grouped based on Nakdong and Dongjin leaves, indicating significant beta diversity differences between the genotypes. In terms of endophytic community composition, Nakdong and Dongjin had different compositions, with some genus abundances increasing and others decreasing in bacteria (Fig 3a) and fungi (Fig 3b). Bacteria had more than 50% of their genera as unidentified, while fungi had almost all the genera names identified. Notably, bacterial genera more abundant in Nakdong were *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Microbacterium*. On the oterh hand, bacterial genera more abundant in Dongjin were *Stenotrophomonas*, *Methylobacterium-Methylorubrum*, and *Pseudomonas*. Among the fungi, genera more abundant in Nakdong were *Alternaria*, *Neosetophoma*, *Cladosporium*, and *Fusarium*, while genera more abundant in Dongjin were *Moesziomyces* and *Sarocladium*. The results show similar alpha diversity but different beta diversity and community composition of specific endophytes between Nakdong and Dongjin.

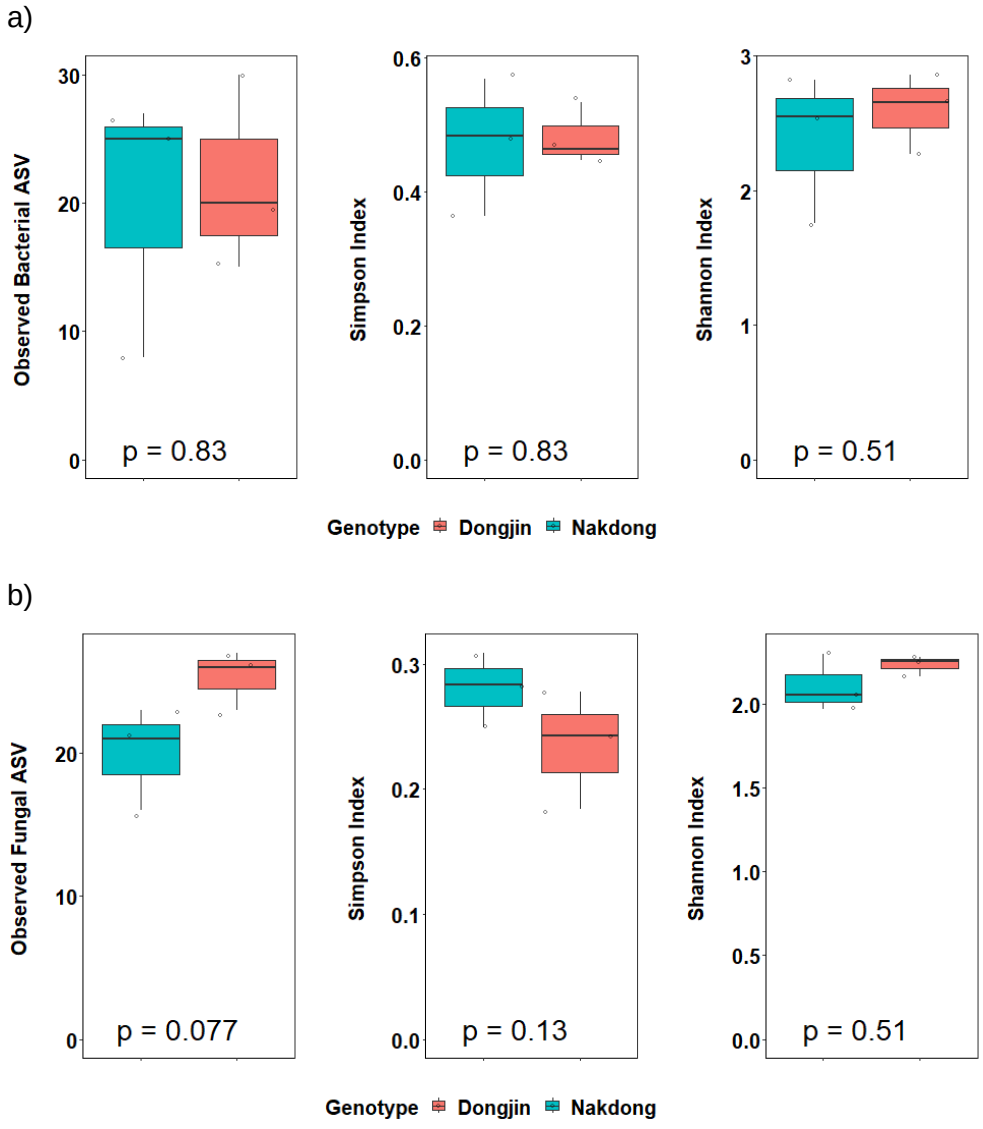


Figure 1. Alpha diversity in Nakdong and Dongjin

Nakdong and Dongjin leaf endophytes were compared based on bacterial alpha diversity (Fig 1a) and fungal alpha diversity (Fig 1b). The p-values for alpha diversity p-values were calculated using the Kruskal-Wallis test. The methods used for assessment included Observed ASV, Simpson index, and Shannon index.

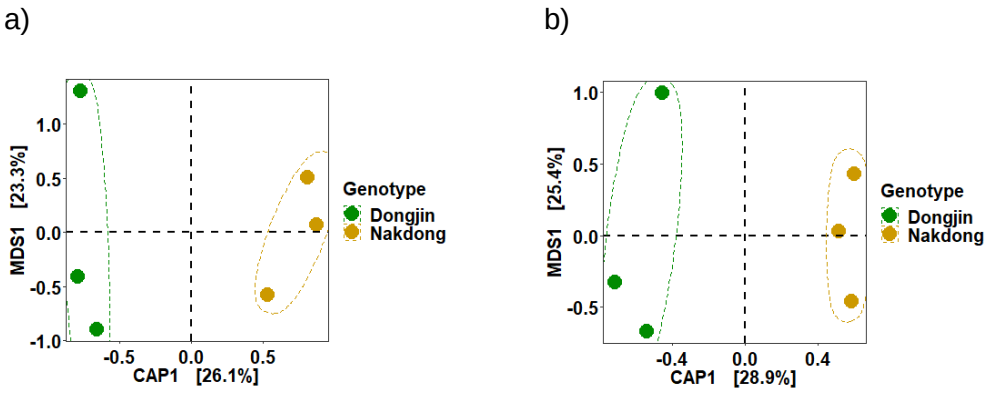


Figure 2. Beta diversity in Nakdong and Dongjin

Nakdong and Dongjin leaf endophytes were compared based on bacterial beta diversity (Fig 2a) and fungal beta diversity (Fig 2b). Beta diversity was assessed using the Canonical analysis of principal coordinates.

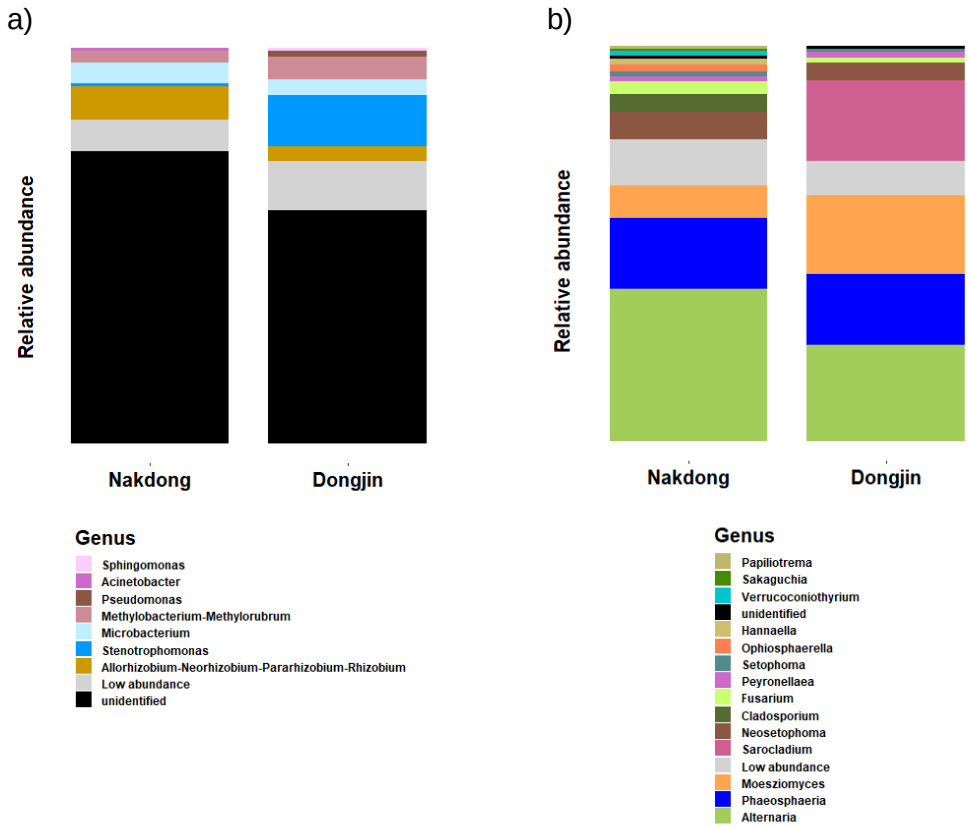


Figure 3. Community composition in Nakdong and Dongjin

Nakdong and Dongjin leaf endophytes were compared based on bacterial community composition (Fig 3a) and fungal community composition (Fig 3b). The community composition illustrates the relative abundance of endophytes, with low abundance (< 0.5%) shown in light gray and unidentified endophytes displayed in black.

II. Different endophytes enrich in Nakdong and *MoHTR1_OX*

Nakdong and *MoHTR1_OX* leaves were compared on their endophyte communities. Alpha diversity did not differ significantly between endophytes of Nakdong and *MoHTR1_OX* leaves for both bacteria (Fig 4a) and fungi (Fig 4b). The observed alpha diversity indices were Observed ASV, Simpson Evenness, and Shannon Diversity. Regarding beta diversity, CAP (Canonical analysis of principal coordinates) analysis showed that bacterial communities (Fig 5a) and fungal communities (Fig 5b) distinctly grouped based on Nakdong and *MoHTR1_OX* leaves, indicating significant beta diversity differences between the genotypes. In terms of endophytic community composition, Nakdong and *MoHTR1_OX* exhibited different compositions, with certain genus abundances increasing and others decreasing in bacteria (Fig 6a) and fungi (Fig 6b). Bacteria had more than 50% of their genera unidentified, while fungi had almost all the genera names identified. Notably, bacterial genera more abundant in Nakdong were *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium, Microbacterium,* and *Stenotrophomonas*. On the other hand, bacterial genera more abundant in *MoHTR1_OX* were *Acinetobacter* and *Pseudomonas*. Among the fungi, genera more abundant in Nakdong were *Moesziomyces, Neosetophoma,* and *Fusarium,* while genera more abundant in *MoHTR1_OX* were *Cladosporium* and *Nigrospora.* Differential abundance analysis revealed that the top ASVs of bacteria and fungi enriched more in *MoHTR1_OX* than in Nakdong (Fig 7). In summary, results demonstrate similar alpha diversity, different beta diversity, different relative abundance of specific

endophytes, and more differentially abundant ASVs in *MoHTR1_OX* compared to Nakdong.

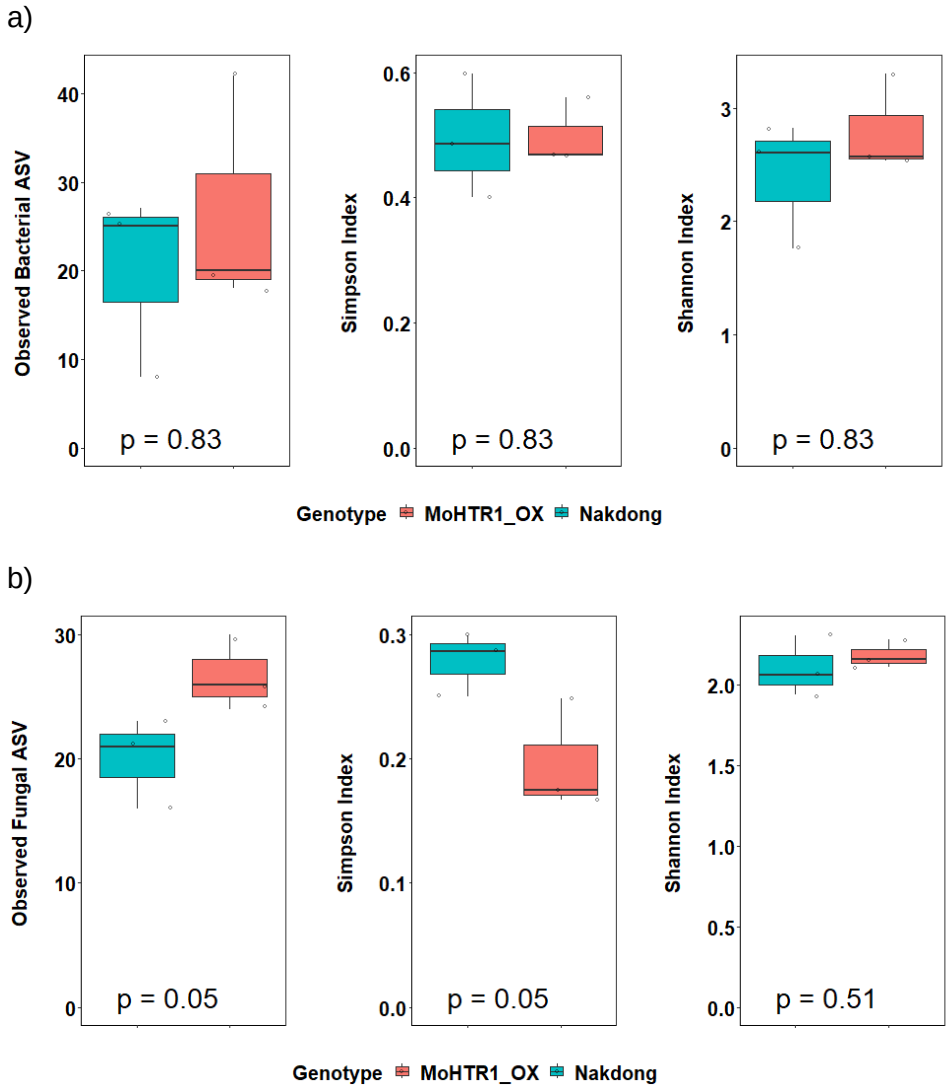


Figure 4. Alpha diversity in Nakdong and *MoHTR1_OX*

Nakdong and *MoHTR1_OX* leaf endophytes were compared based on bacterial alpha diversity (Fig 4a) and fungal alpha diversity (Fig 4b). The p-values for alpha diversity were calculated using the Kruskal-Wallis test. The methods used for assessment included Observed ASV, Simpson index, and Shannon index.

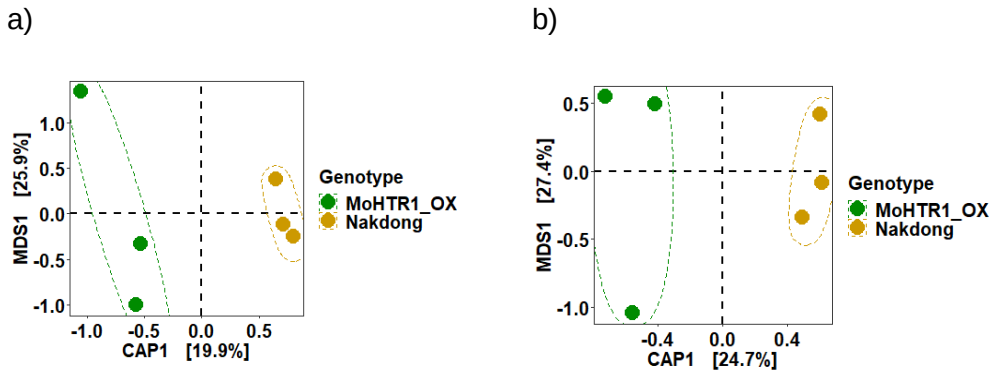


Figure 5. Beta diversity in Nakdong and *MoHTR1_OX*

Nakdong and *MoHTR1_OX* leaf endophytes were compared based on bacterial beta diversity (Fig 5a) and fungal beta diversity (Fig 5b). Beta diversity was assessed using Canonical analysis of principal coordinates.

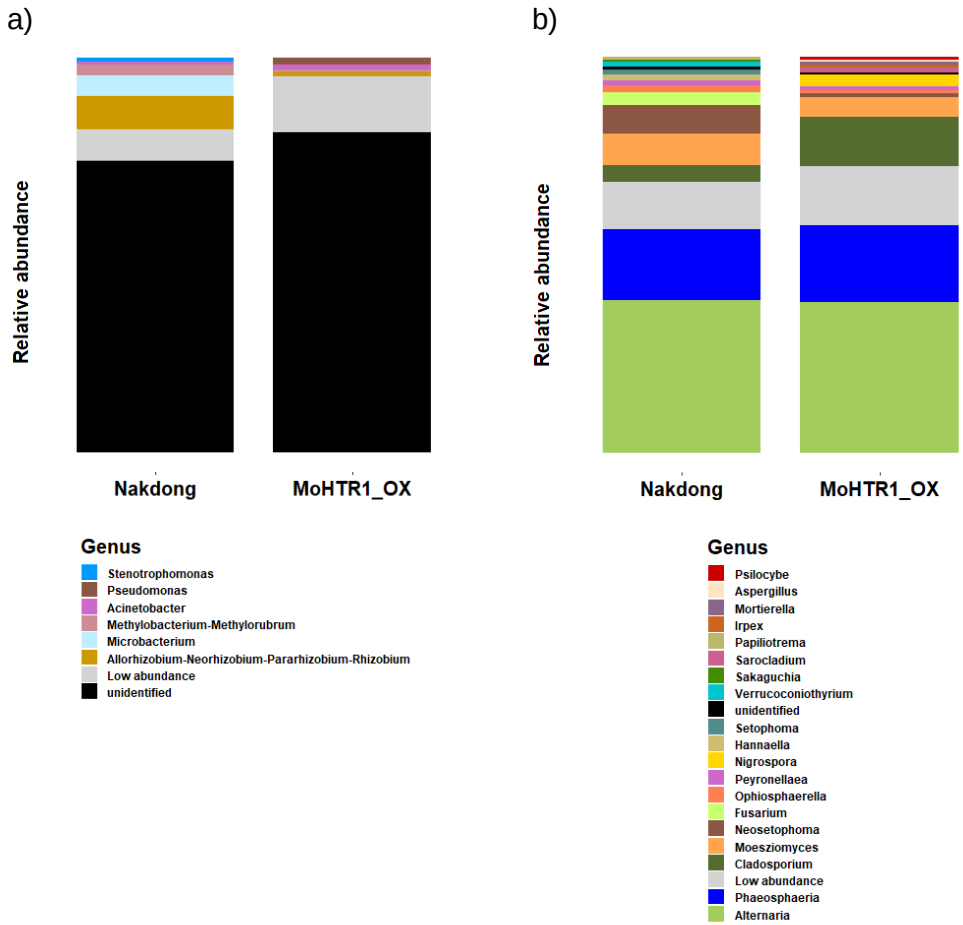
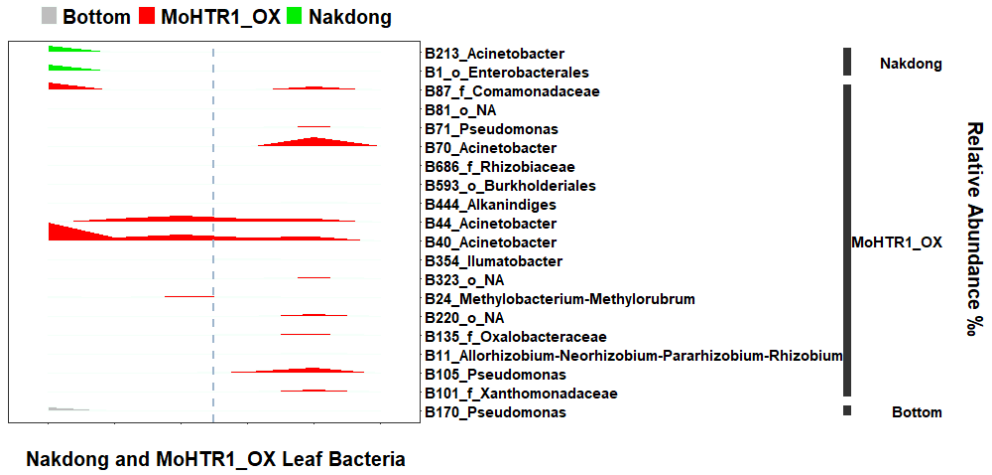


Figure 6. Diversity and abundance in Nakdong and *MoHTR1_OX*

Nakdong and *MoHTR1_OX* leaf endophytes were compared based on bacterial community composition (Fig 6a) and fungal community composition (Fig 6b). The community composition illustrates the relative abundance of endophytes, with endophytes having a low abundance (< 0.5%) shown in light gray and unidentified endophytes displayed in black.

a)



b)

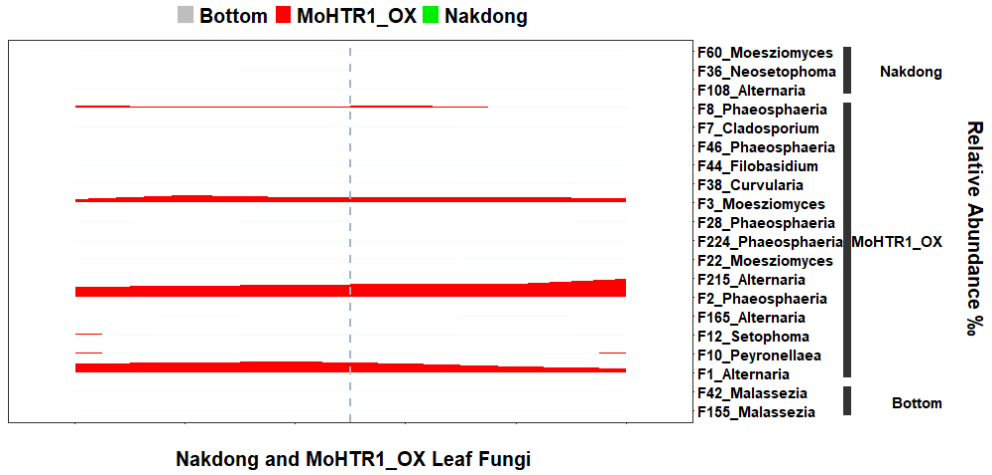


Figure 7. Differential abundance in Nakdong and *MoHTR1_OX*

Nakdong and *MoHTR1_OX* leaf endophytes were compared based on bacterial differential abundance (Fig 7a) and fungal differential abundance (Fig 7b). ASVs enriched in Nakdong are represented in green, while ASVs enriched in *MoHTR1_OX* are shown in red.

III. Different endophytes enrich in Dongjin and *OsMORE1a*

Leaves of Dongjin and *OsMORE1a* were compared based on their endophyte communities. Alpha diversity did not differ significantly between endophytes of Dongjin and *OsMORE1a* leaves for both bacteria (Fig 8a) and fungi (Fig 8b). The observed alpha diversity indices were Observed ASV, Simpson Evenness, and Shannon Diversity. Regarding beta diversity, CAP (Canonical analysis of principal coordinates) analysis showed that bacterial communities (Fig 9a) and fungal communities (Fig 9b) distinctly grouped based on Dongjin and *OsMORE1a* leaves, indicating significant beta diversity differences between the genotypes. In terms of endophytic community composition, Dongjin and *OsMORE1a* exhibited different compositions, with certain genus abundances increasing and others decreasing in bacteria (Fig 10a) and fungi (Fig 10b). Bacteria had about 50% of its genera unidentified, while fungi had almost all the genera identified. Notably, bacterial genera more abundant in Dongjin were *Microbacterium* and *Stenotrophomonas*. On the other hand, bacterial genera more abundant in *OsMORE1a* were *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Methylobacterium-Methylorubrum*. Among the fungi, genera more abundant in Dongjin were *Alternaria*, *Moesziomyces*, and *Neosetophoma*, while genera more abundant in *OsMORE1a* were *Fusarium* and *Mortierella*. Differential abundance analysis revealed that the top ASVs of bacteria and fungi were enriched more in Dongjin than in *OsMORE1a* (Fig 11). In summary the results demonstrate similar alpha diversity, different beta diversity, different relative abundance of

specific endophytes, and more differentially abundant ASVs in Dongjin when comparing Dongjin and *OsMORE1a*.

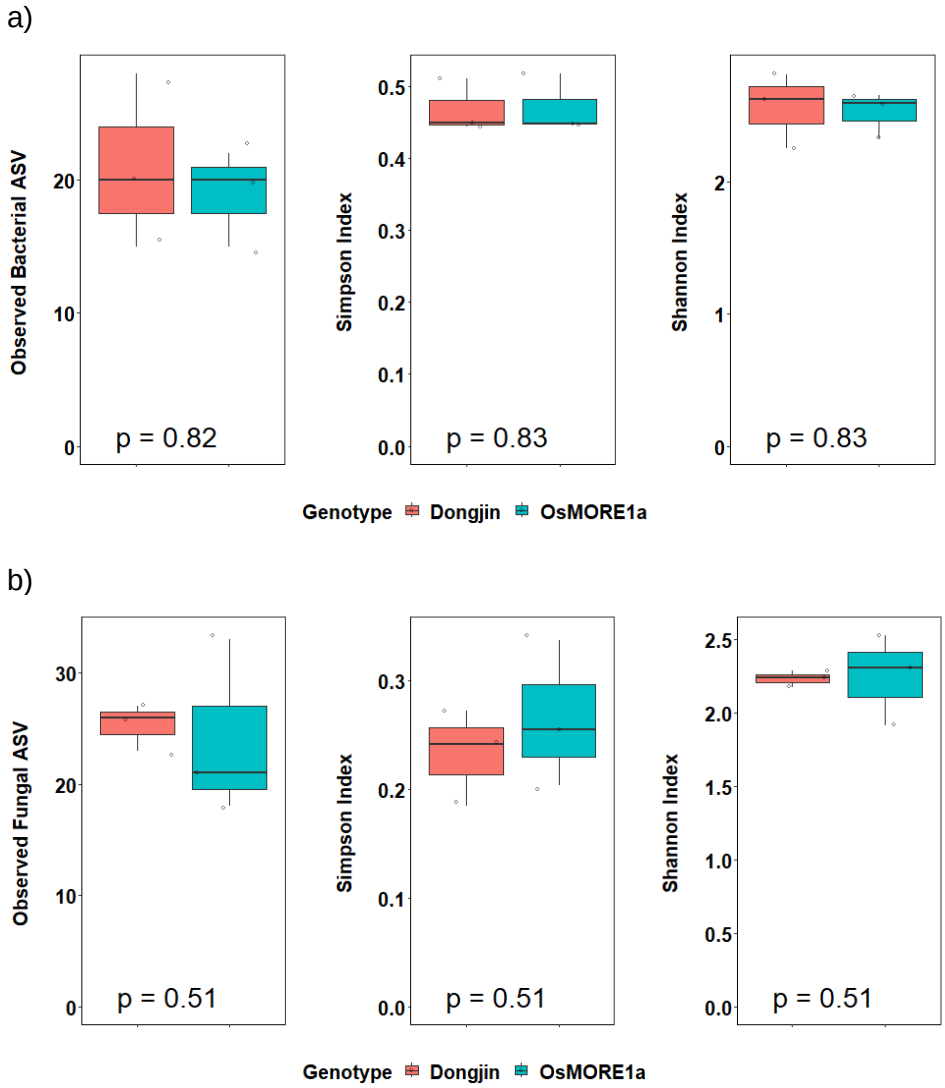


Figure 8. Alpha diversity in Dongjin and *OsMORE1a*

Dongjin and *OsMORE1a* leaf endophytes were compared based on bacterial alpha diversity (Fig 8a) and fungal alpha diversity (Fig 8b). The p-values for alpha diversity were calculated using the Kruskal-Wallis test. The methods used for assessment included Observed ASV, Simpson index, and Shannon index.

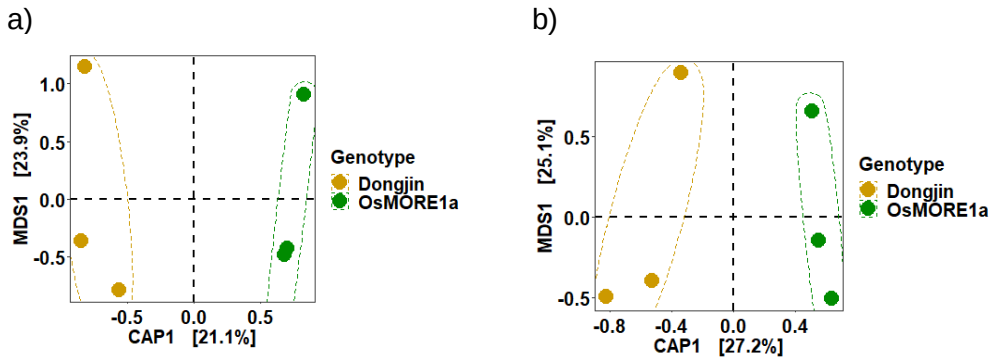


Figure 9. Beta diversity in Dongjin and *OsMORE1a*

Dongjin and *OsMORE1a* leaf endophytes were compared based on bacterial beta diversity (Fig 9a) and fungal beta diversity (Fig 9b). Beta diversity was assessed using Canonical analysis of principal coordinates.

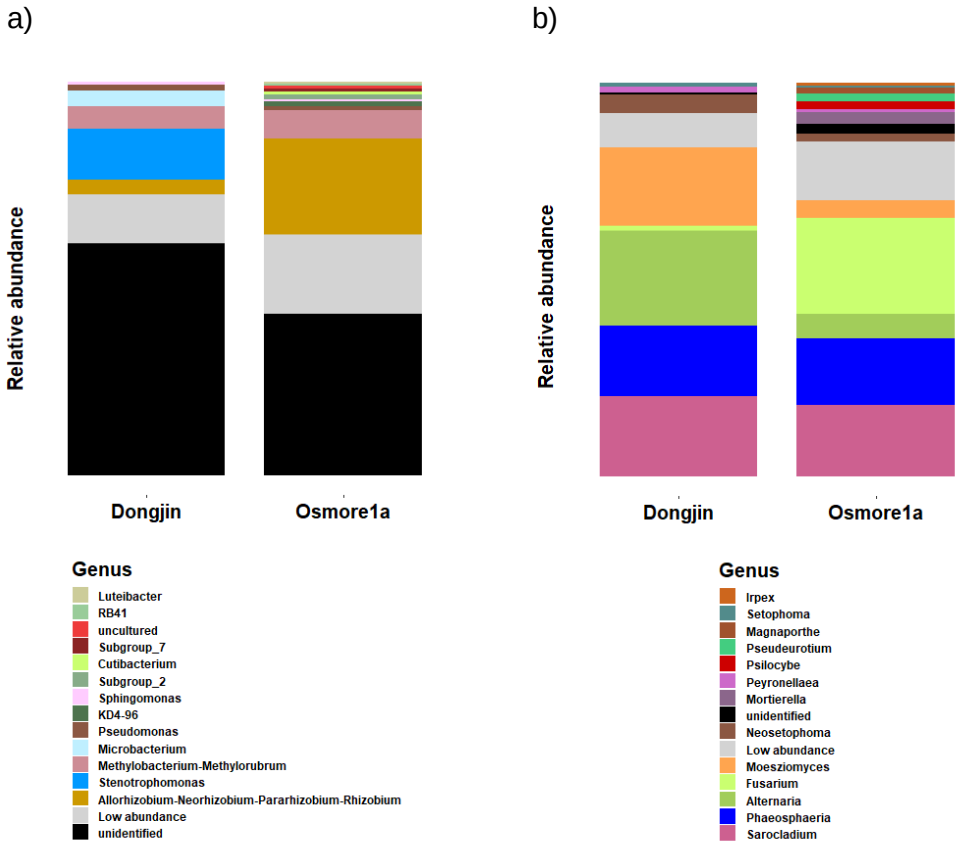
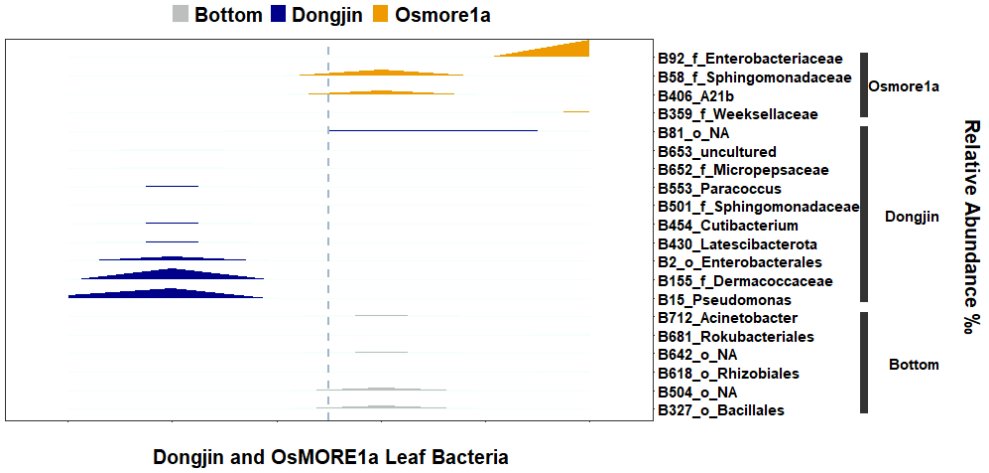


Figure 10. Community composition in Dongjin and *OsMORE1a*

Dongjin and *OsMORE1a* leaf endophytes were compared based on bacterial community composition (Fig 10a) and fungal community composition (Fig 10b). The community composition illustrates the relative abundance of endophytes, with endophytes having a low abundance (< 0.5%) shown in light gray, and unidentified endophytes displayed in black.

a)



b)

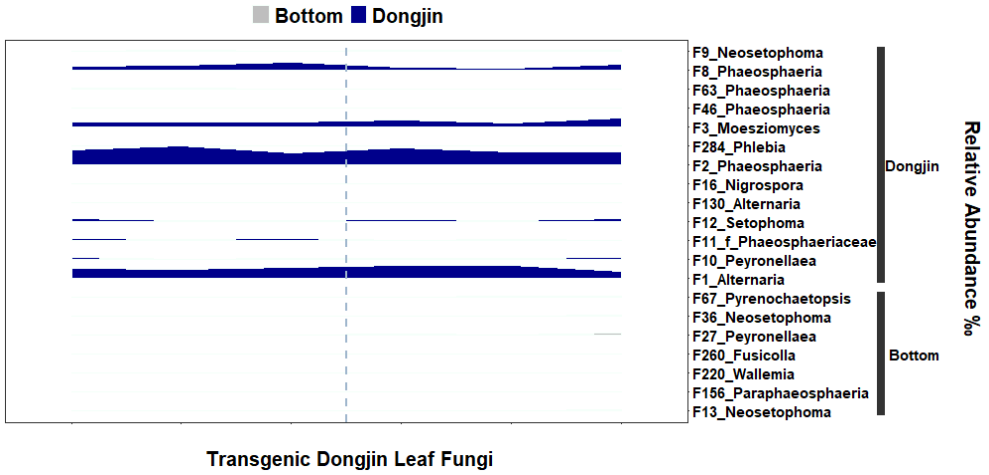


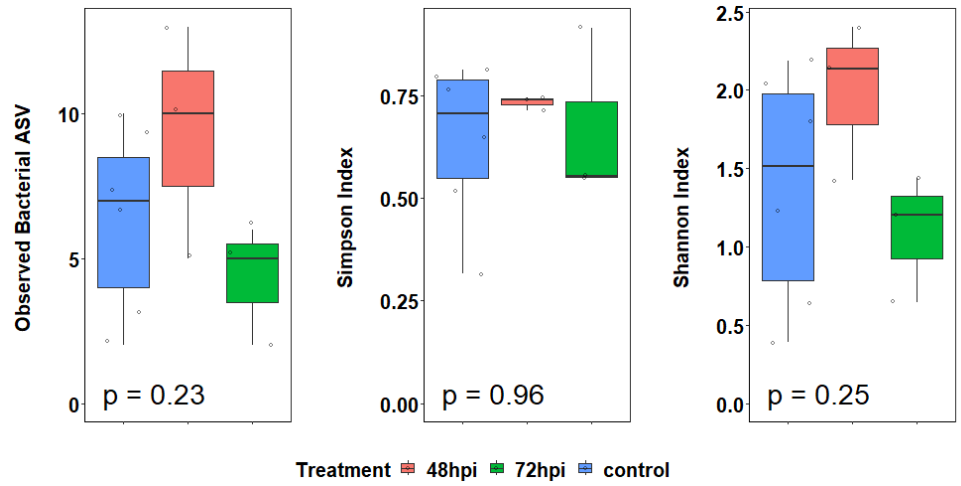
Figure 11. Differential abundance in Dongjin and *OsMORE1a*

Dongjin and *OsMORE1a* leaf endophytes were compared based on bacterial differential abundance (Fig 11a) and fungal differential abundance (Fig 11b). ASVs enriched in Dongjin are represented in blue, while ASVs enriched in *OsMORE1a* are shown in orange

IV. Different bacteria enrich in *M. oryzae*-infected leaves

Leaves of *M. oryzae*-infected leaves were compared based on their endophyte communities. Alpha diversity did not differ significantly between endophytes of uninfected and infected leaves for both Nakdong (Fig 12a) and Dongjin (Fig 12b). The observed alpha diversity indices were Observed ASV, Simpson Evenness, and Shannon Diversity. Regarding beta diversity, CAP (Canonical analysis of principal coordinates) analysis showed in Nakdong (Fig 13a) and Dongjin (Fig 13b) that uninfected, infected 48hpi, and infected 72hpi leaves grouped separately, indicating significant beta diversity differences between the treatments. In terms of endophytic community composition, uninfected and infected leaves had different compositions, with some genus abundances increasing and others decreasing in Nakdong (Fig 14a) and Dongjin (Fig 14b). Notably, a bacterial genus that increased in Nakdong infected treatments from uninfected to 48hpi, and 72hpi was *Pseudomonas*, while *Enhydrobacter* decreased. In Dongjin-infected treatments, *Pseudomonas* and *Cutibacterium* increased, while *Bacillus* decreased. However, differential abundance analysis did not reveal whether the top bacterial ASVs enriched in uninfected or infected leaves, as trends varied when comparing uninfected leaves with 48hpi leaves and 72hpi (Fig 15-16). In conclusion, the results show similar alpha diversity, different beta diversity, different relative abundance of specific endophytes, and non-differentially abundant ASVs towards uninfected and infected treatments.

a)



b)

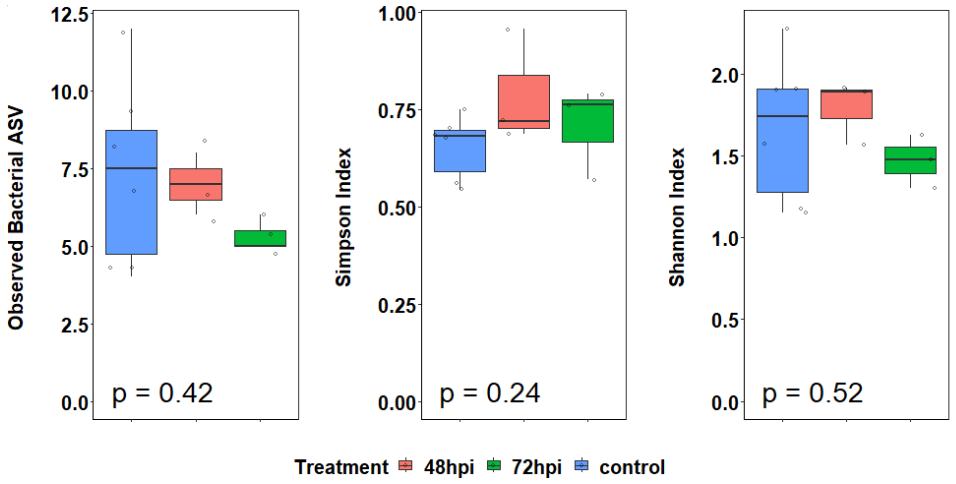
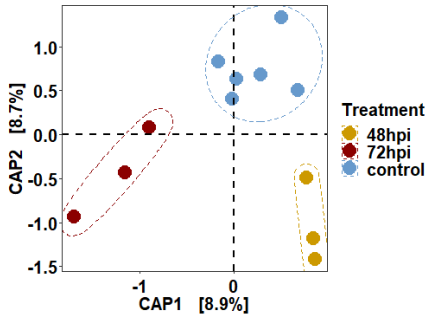


Figure 12. Alpha diversity in *M. oryzae*-infected leaves

Bacterial endophytes of *M. oryzae* infected leaves were compared based on Nakdong alpha diversity (Fig 12a) and Dongjin alpha diversity (Fig 12b). The p-values for alpha diversity were calculated using the Kruskal-Wallis test. The methods used for assessment included Observed ASV, Simpson index, and Shannon index.

a)



b)

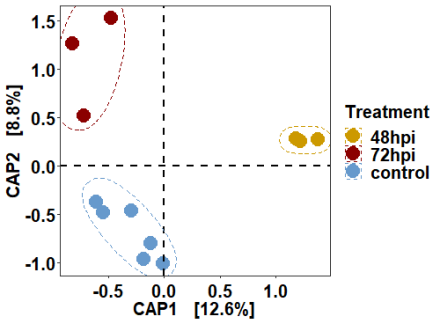


Figure 13. Beta diversity in *M. oryzae*-infected leaves

Bacterial endophytes of *M. oryzae* infected leaves were compared based on Nakdong beta diversity (Fig 13a) and Dongjin beta diversity (Fig 13b). Beta diversity was assessed using Canonical analysis of principal coordinates.

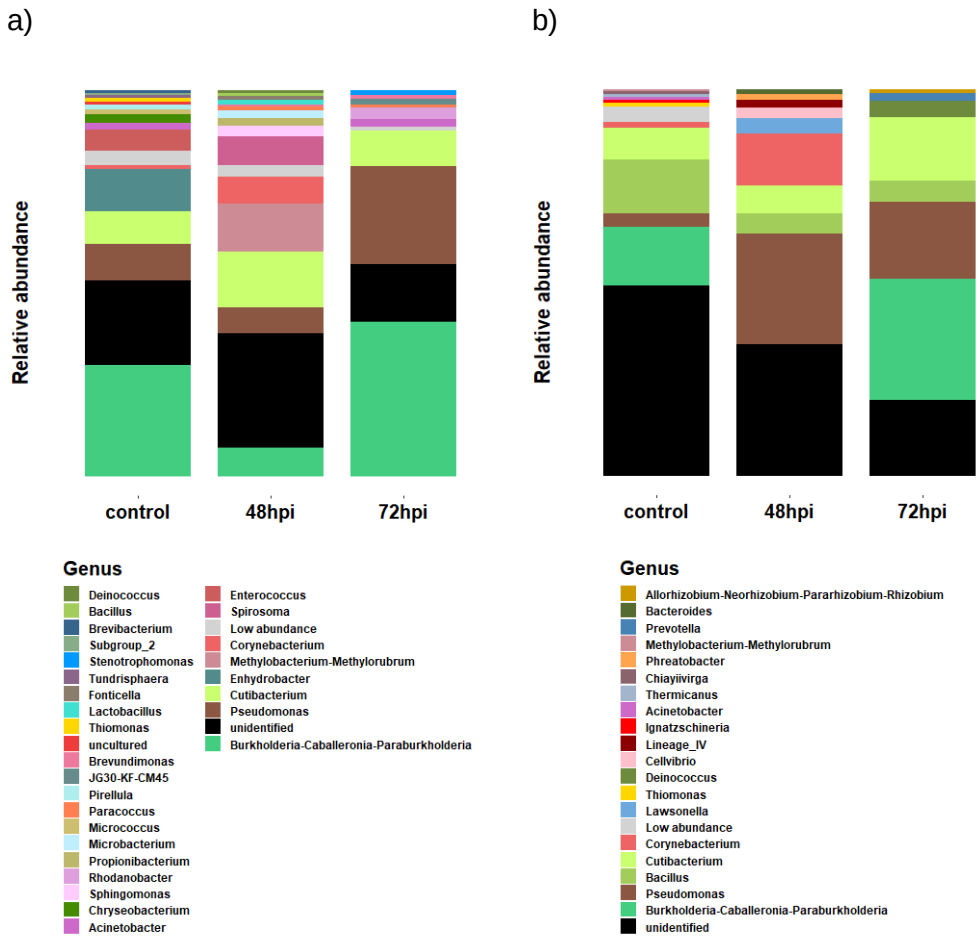
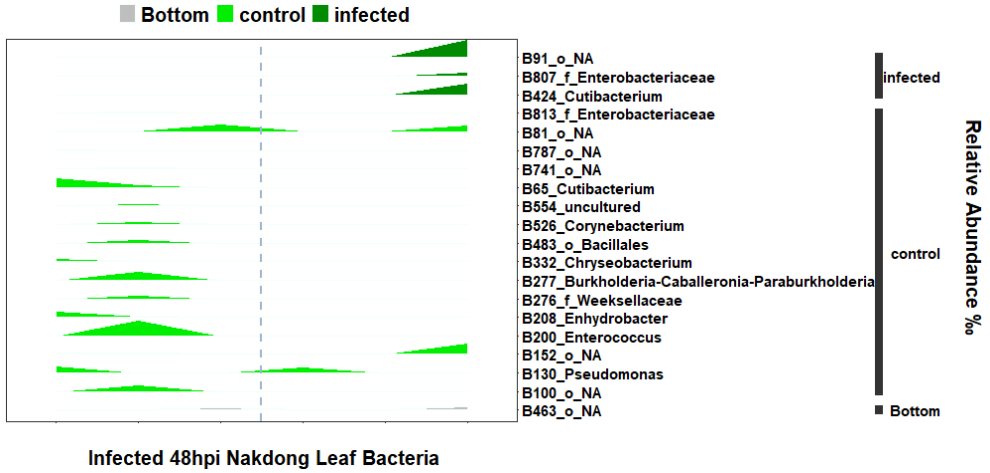


Figure 14. Community composition in *M. oryzae*-infected leaves

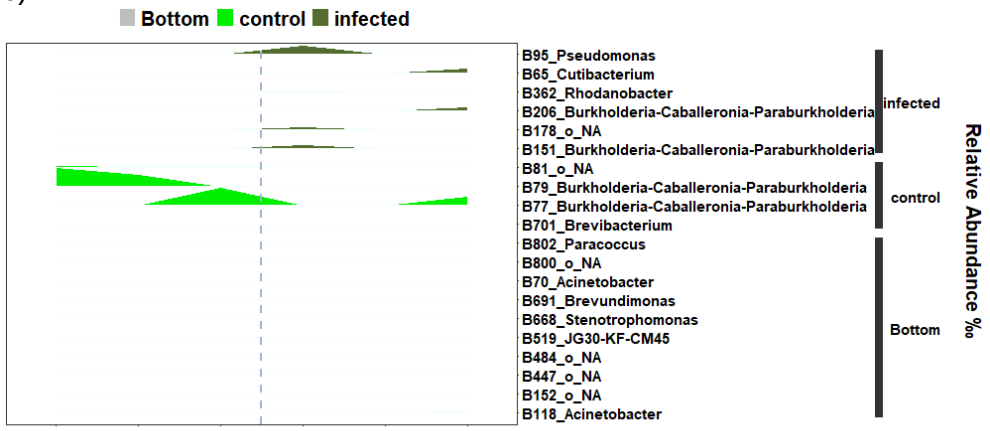
Bacterial endophytes of *M. oryzae* infected leaves were compared based on the Nakdong community composition (Fig 14a) and Dongjin community composition (Fig 14b). The community composition illustrates the relative abundance of endophytes, with endophytes having a low abundance (< 0.5%) shown in light gray, and unidentified endophytes displayed in black.

a)



Infected 48hpi Nakdong Leaf Bacteria

b)

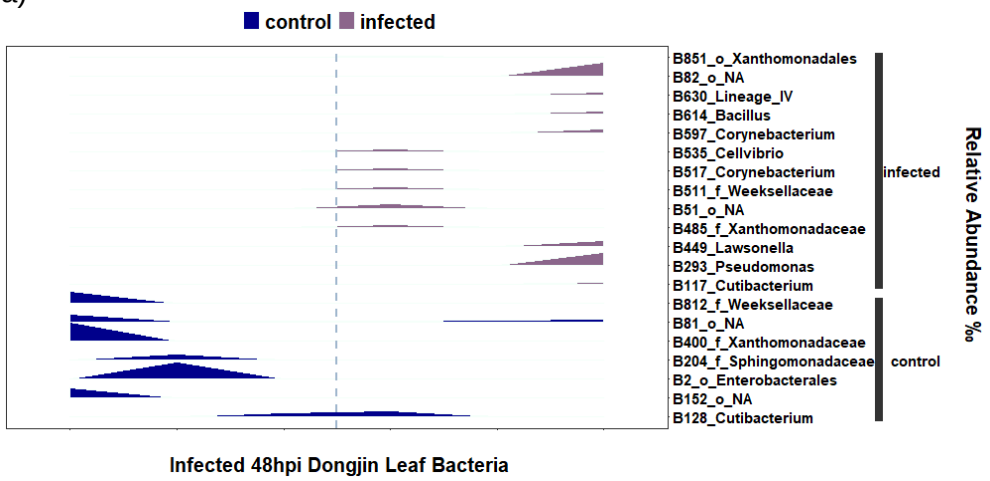


Infected 72hpi Nakdong Leaf Bacteria

Figure 15. Differential abundance in *M. oryzae*-infected Nakdong leaves

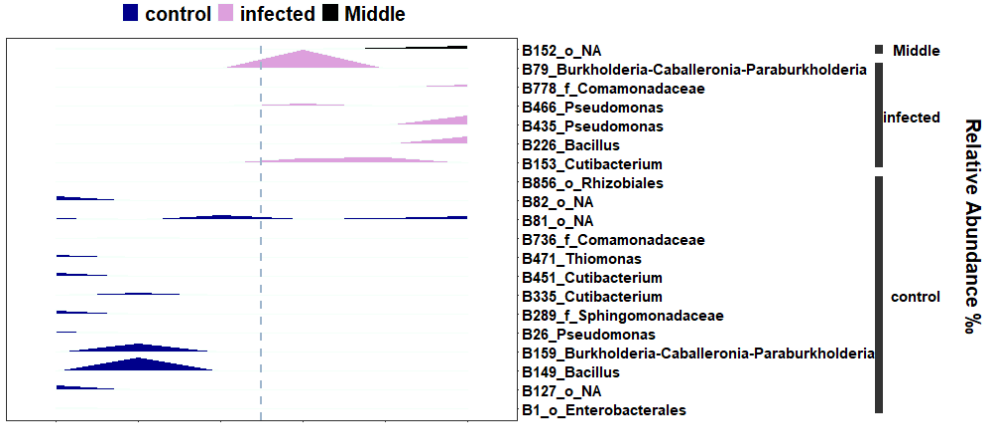
Bacterial endophytes of *M. oryzae*-infected leaves were compared based on Nakdong differential abundance for 48 hpi (Fig 15a) and Nakdong differential abundance for 72 hpi (Fig 15b). ASVs enriched in the infected samples are represented in dark green, while ASVs enriched in the uninfected samples are shown in light green.

a)



Infected 48hpi Dongjin Leaf Bacteria

b)



Infected 72hpi Dongjin Leaf Bacteria

Figure 16. Differential abundance in *M. oryzae*-infected Dongjin leaves

Bacterial endophytes of *M. oryzae*-infected leaves were compared based on Dongjin differential abundance for 48 hpi (Fig 16a) and Dongjin differential abundance for 72 hpi (Fig 16b). ASVs enriched in the infected samples are represented in pink or purple, while ASVs enriched in the uninfected samples are shown in blue.

V. Fungi were central in the co-occurrence network of endophytes

A co-occurrence network of all leaf endophytes from this study investigated the microbial relations between ASVs. There were 26 nodes and 119 edges with a threshold set as correlations of > 0.3 and < −0.3 (p-value < 0.05) (Fig 17a). ASVs with the highest between centrality, closeness centrality, and degree centrality were observed (Zhang and Luo, 2017) (Fig 17b). ASVs starting with "B" are bacteria, while ASVs starting with "F" are fungi. ASVs within the highest 20% of between centrality were F3_Moesziomyces, B152_o_NA, F1_Alternaria, and F2_Phaeosphaeria. Betweenness centrality measures how often a node is in the path between two other nodes. Three out of four ASVs with the highest between centrality were fungi. ASVs within the highest 20% closeness centrality were B24_Methylobacterium-Methylorubrum, F60_Moesziomyces, F42_Malassezia, and B40_Acinetobacter. Closeness centrality measures how many edges it takes for a node to travel to all other nodes. ASVs within the highest 20% of degree centrality were F3_Moesziomyces, F1_Alternaria, F2_Phaeosphaeria, and F8_Phaeosphaeria. Degree centrality is the number of edges radiating from a node. The results show that F3_Moesziomyces, F1_Alternaria, and F2_Phaeosphaeria are at the top 20% in both between centrality and degree centrality, which classifies these three fungal ASVs as hub ASVs for the leaf endophytic microbiome.

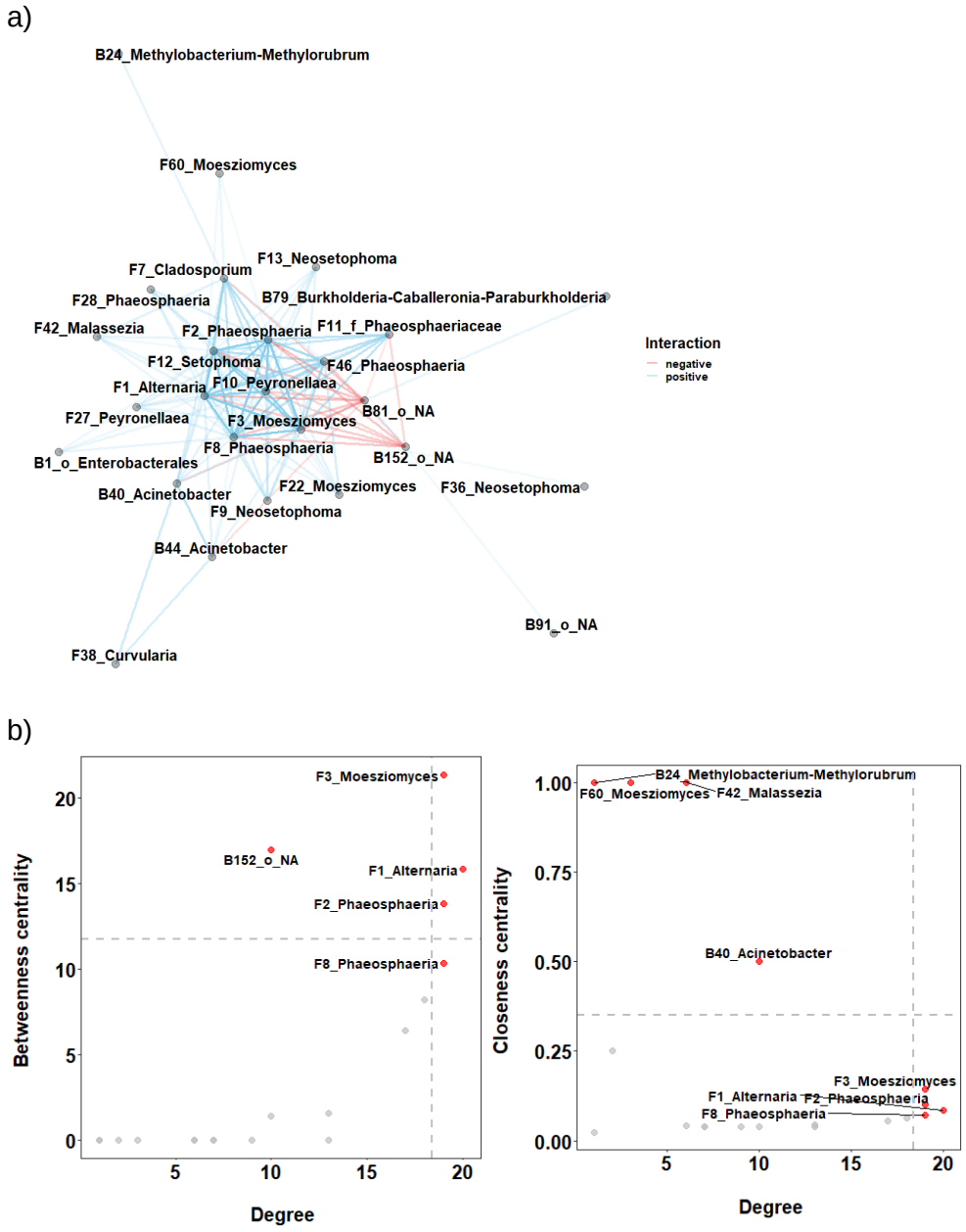


Figure 17. Co-occurrence network analysis for all leaves

Endophytes of all leaf samples were analyzed based on the overall co-occurrence network (Fig 17a), with an analysis on the thresholds of between centrality, closeness centrality, and degree centrality (Fig 17b). Dashed lines delineate nodes in red dots, representing the top 20% of each threshold.

DISCUSSION

The plant pathobiome has been studied in various situations to understand how the microbiome responds to dysbiosis as a pathobiome. One of the most critical aspects of the microbiome is diversity. For example, a closely related study on rice investigated the rhizosphere and root endosphere microbiome diversity of *M. oryzae* infection and transgenic rice having an R gene, *Piz-t* (Tian et al., 2021). Their results showed that the root endosphere bacterial alpha diversity increased in infected samples, while the root endosphere fungal alpha diversity decreased in infected samples, which was different from our research (Fig 4, 8) where there were no statistically significant differences between leaf endophyte treatments. However, the beta diversity of root endophytes from their study and the leaf endophytes from our study (Fig 5, 9) were both clearly separately clustered. The co-occurrence network analysis of the whole leaf endophytic microbiome (Fig 17) yielded similar results, as most correlations between ASVs were also positive for root endosphere and rhizosphere (Tian et al., 2021). Another study on rice blast infection of different plant parts revealed that the alpha diversity of the plant microbiome for bacteria and fungi does not change with infection for bulk soil, rhizosphere, root endosphere, and leaf endosphere (Dastogeer et al., 2022). The sampling method differed from our study since they sampled from field conditions, which should contain more noise in the data than lab-grown samples. The beta diversity included more noise since compared samples did not have clear distinctions with each other. A diversity study

on the endophyte pathobiome of maize with sugarcane mosaic virus discovered that the virus invasion lowered the root endosphere bacterial alpha diversity, unlike the rhizosphere alpha diversity that did not experience alpha diversity shifts (Liu et al., 2023). Their beta diversity of the root endosphere experienced a statistically significant shift, while the rhizosphere was not significant. A study on wheat infected with *Zymoseptoria tritici* revealed that the bacterial alpha diversity was lower for wheat with more resistance toward the disease than the susceptible variety (Seybold et al., 2020), which was not observed in our study comparing wildtype rice with transgenic rice (Fig 4a, 8a).

When comparing the interkingdom influence of bacteria and fungi on the leaf endosphere microbiome, fungi have been found to play a significant role, as three fungi of the genus *Alternaria, Phaeosphaeria*, and *Moesziomyces* have been identified as hub microbes in this study (Fig 17b). One closely related study compared the co-occurrence network in the rhizosphere and root endosphere, but it did not examine the interkingdom level for fungi and bacteria (Tian et al., 2021). Therefore, their interactions between fungi and bacteria were not determined. However, another study did look at the interkingdom level, but it focused on the positive and negative relationships between *M. oryzae* and the microbiome, not within the microbiome (Dastogeer et al., 2022). Their networks found that bacteria were far more interactive with *M. oryzae* compared to fungi, and none of our hub microbe members, which were *Alternaria, Phaeosphaeria,* and *Moesziomyces* (Fig 17b), were present in their network analysis as interacting with *M. oryzae*. Therefore, our three hub microbes, *Alternaria, Phaeosphaeria*, and *Moesziomyces*,

were important in the microbiome but not with the pathogen *M. oryzae*.

Most plant pathobiome studies have focused on the rhizosphere and soil due to more accessible sampling compared to endophyte samples. Some studies have compared the alpha diversity of the rice rhizosphere and endosphere and found higher bacterial alpha diversity in the rhizosphere but higher fungal alpha diversity in the endosphere (Rodriguez et al., 2009; Wang et al., 2016; Tian et al., 2021; Dastogeer et al., 2022). Rice blast infection influenced the rhizosphere bacterial microbiome but not the fungal microbiome in the study by Dastogeer et al. (2022). They found that rice blast infection affected the genera *Streptomyces*, *Burkholderia*, and *Caulobacter* in the roots. *Burkholderia* was also abundant in our rice blast infection experiments (Fig 14), but without apparent enrichment towards rice blast-infected and uninfected samples. A random forest analysis comparing bacteria of *M. oryzae*-infected leaves and uninfected leaves showed that symptomatic leaves had consistently enriched abundances of *Rhizobium, Pedobacter, Methylobacterium*, and *Stenotrophomonas* (Dastogeer et al., 2022). On the other hand, decreased genera in infected samples included *Paenibacillus* and *Enterobacter*. *Methylobacterium* and *Stenotrophomonas* were also present in our study as being affected by transgenic rice samples (Fig 6a, 10a).

One notable genus in this study is *Pseudomonas,* enriched in samples infected with *M. oryzae* (Fig 14). *Pseudomonas spp.* is a diverse genus encompassing one of the broadest ecological niches, ranging from pathogens to beneficial bacteria, and from animals to plants (Silby et al., 2010). *Pseudomonas* of the same species can cause plant diseases and simultaneously act as biocontrol

agents or facilitate plant growth promotion, depending on the strain level. Regarding *Pseudomonas spp*. interacting with *M. oryzae, Pseudomonas putida* was found to exert a strong inhibitory effect on *M. oryzae* using cyclic lipopeptides (Omoboye et al., 2019). Other rice blast biocontrol *Pseudomonas spp*. include *P. fluorescens* and *P. alcaliphiphila* (Krishnamurthy and Gnanamanickam, 1998; Zeng et al., 2023). The increase in *Pseudomonas spp.* in our study may suggest that these endophytes interact with the rice blast pathobiome.

In the case of *MoHTR1_OX, OsMORE1a*, and rice blast infection, the pathobiome is related to the ambivalent immunity of necrotrophic and (hemi)biotrophic pathogens (Kim et al., 2020; Kim et al., 2022). *MoHTR1_OX,* when compared to Nakdong, increases the plant susceptibility towards *Xanthomonas oryzae* pv. *oryzae*, a hemibiotrophic bacterial pathogen, but increases resistance in *Cochliobolus miyabeanus,* a necrotrophic fungal pathogen (Kim et al., 2020). The opposite works for *OsMORE1a* compared to Dongjin by increasing rice resistance towards *X. oryzae* pv. *oryzae*, the hemibiotrophic bacterial pathogen, but enhancing susceptibility towards *C. miyabeanus*, the necrotrophic fungal pathogen (Kim et al., 2022).

Among the related hormones is salicylic acid, which favors defense against necrotrophic pathogens but endangers the plant against biotrophic or hemibiotrophic pathogens (Spoel et al., 2007). *MoHTR1_OX* had higher salicylic acid production than its wildtype, Nakdong, while *OsMORE1a* had lower salicylic acid production than its wildtype, Dongjin (Kim et al., 2020; Kim et al., 2022). Regarding the random forest analysis results comparing transgenic rice leaves and wildtype rice

leaves (Fig 7, 11), more differentially abundant ASVs enriched in *MoHTR1_OX* than Nakdong and Dongjin than *OsMORE1a*, which were salicylic acid richer counterparts. This suggests that more endophytes prefer the biotrophic pathogen-favored environment, as indicated by higher salicylic acid levels in the plants. In infection experiments comparing infected and uninfected samples, efforts were made to observe similar patterns when the hemibiotroph *M. oryzae* was in its biotrophic phase at 48 hours post-inoculation and the necrotrophic phase at 72 hours post-inoculation. However, representative differences in the random forest analyses were not found (Fig 15, 16). A study done on salicylic acid and jasmonic acid in the rice root endosphere found that in transgenic varieties, lower salicylic acid production due to mutation lowered *X. oryzae* pv. *oryzae* colonization in the root, which was opposite for leaves, showing more colonization with lower salicylic acid production (Chen et al., 2020). They also noted that salicylic acid is related to pathogen defense, while jasmonic acid is more related to beneficial endophyte root colonization.

In conclusion, some uniform trends were observed across different experiments. The alpha diversity was not significantly different for both genetically altered immunity experiments, comparing transgenic plants and wildtype plants, and infection experiments comparing infected and uninfected plants. However, the beta diversity was significantly different between transgenic and wildtype plants, showing clear separation, and also between infected and uninfected plants. Individual genera and ASVs were differentially enriched based on community composition and differential abundance analyses. The results from this research can

be valuable for future studies concerning endophytes that respond to pathogen infection and plant genetic manipulation. This may help to determine the tripartite relationship between rice, *M. oryzae*, and the endophytes. Overall, this study sheds light on the complex interactions between the plant pathobiome, genetic immunity, and endophytic microbiome, providing important insights into the dynamic processes influencing plant health and disease resistance. Further research in this area will undoubtedly contribute to our understanding of plant-microbe interactions and their implications for agriculture and plant health management.

LITERATURE CITED

Bass, D., Stentiford, G.D., Wang, H.C., Koskella, B., and Tyler, C.R. 2019. The pathobiome in animal and plant diseases. Trends in Ecology & Evolution 34:996-1008.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., and Caporaso, J.G. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6:90.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T. Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., Van Der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., Von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D.,

Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., and Caporaso, J.G. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37:852-857.

Busby, P.E., Soman, C., Wagner, M.R., Friesen, M.L., Kremer, J., Bennett, A., Morsy, M., Eisen, J.A., Leach, E.L., and Dangl, J.L. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. PLoS Biology 15:e2001793.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods 13:581-583.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., and Knight, R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences, U.S.A. 108:4516-4522.

Chen, X., Marszałkowska, M., and Reinhold-Hurek, B. 2020. Jasmonic acid, not salicyclic acid restricts endophytic root colonization of rice. Frontiers in Plant Science 10:1758.

Compant, S., Clément, C., and Sessitsch, A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biology and Biochemistry 42:669-678.

Csardi, G., and Nepusz, T. 2006. The igraph software package for complex network research. InterJournal, complex systems 1695:1-9.

Dastogeer K.M., Tumpa, F.H., Sultana, A., Akter, M.A., and Chakraborty, A. 2020. Plant microbiome-an account of the factors that shape community composition and diversity. Current Plant Biology 23:100161.

Dastogeer, K.M., Yasuda, M., and Okazaki, S. 2022. Microbiome and pathobiome analyses reveal changes in community structure by foliar pathogen infection in rice. Frontiers in Microbiolgy 13:949152.

Defazio J., Fleming, I.D., Shakhsheer, B., Zaborina, O., and Alverdy, J.C. 2014.

The opposing forces of the intestinal microbiome and the emerging pathobiome. Surgical Clinics 94:1151-1161.

Doonan, J., Denman, S., Pachebat, J.A., and McDonald, J.E. 2019. Genomic analysis of bacteria in the Acute Oak Decline pathobiome. Microbial Genomics 5:e000240.

Jakuschkin, B., Fievet, V., Schwaller, L., Fort, T., Robin, C., and Vacher, C. 2016. Deciphering the pathobiome: intra- and interkingdom interactions involving the pathogen erysiphe alphitoides. Microbial Ecology 72: 870-880.

Kim, S., Kim, C.Y., Park, S.Y., Kim, K.T., Jeon, J., Chung, H., Choi, G., Kwon, S., Choi, J., Jeon, J., Jeon, J.-S., Khang, C.H., Kang, S., and Lee, Y.H. 2020. Two nuclear effectors of the rice blast fungus modulate host immunity via transcriptional reprogramming. Nature Communications 11:5845.

Kim, C.Y., Park, J.Y., Choi, G., Kim, S., Vo, K.T.X., Jeon, J.S., Kang, S., and Lee, Y.H. 2022. A rice gene encoding glycosyl hydrolase plays contrasting roles in immunity depending on the type of pathogens. Molecular Plant Pathology 23:400-416.

Kirtphaiboon S., Humphries, U., Khan, A., and Yusuf, A. 2021. Model of rice blast disease under tropical climate conditions. Chaos, Solitons & Fractals 143:110530.

Krezalek M.A., DeFazio, J., Zaborina, O., Zaborin, A., and Alverdy, J.C. 2016. The shift of an intestinal “microbiome” to a “pathobiome” governs the course and outcome of sepsis following surgical injury. Shock 45:475-482.

Krishnamurthy, K., and Gnanamanickam, S.S. 1998. Biological control of rice blast by *Pseudomonas fluorescens* strain Pf 7–14: evaluation of a marker gene and formulations. Biological Control 13:158-165.

Kurtz, Z., Mueller, C., Miraldi, E., and Bonneau, R. 2023. SpiecEasi: sparse inverse covariance for ecological statistical inference. R package Version 1.1.2. https://CRAN.R-project.org/package=SpiecEasi.

Laluk, K., and Mengiste, T. 2010. Necrotroph attacks on plants: wanton destruction

or covert extortion? The Arabidopsis Book 8:e0136.

Liaw, A., and Wiener, M. 2002. Classification and regression by randomForest. R News 2:18-22.

Liu, W., Cui, X., Wang, X., Shen, C., Ji, L., Zhang, M., Wong, M.H., Zhang, J., and Shan, S. 2023. Sugarcane mosaic virus reduced bacterial diversity and network complexity in the maize root endosphere. mSystems: https://doi.org/10.1128/msystems.00198-23.

Lu, L., Liu, Y., and Zhang, Z. 2020. Global characterization of GH10 family xylanase genes in *Rhizoctonia cerealis* and functional analysis of xylanase RcXYN1 during fungus infection in wheat. International Journal of Molecular Sciences 21:1812.

Lundberg, D.S., Yourstone, S., Mieczkowski, P., Jones, C.D., and Dangl, J.L. 2013. Practical innovations for high-throughput amplicon sequencing. Nature Methods 10:999-1002.

Mannaa, M., and Seo, Y.S. 2021. Plants under the attack of allies: moving towards the plant pathobiome paradigm. Plants 10:125.

McMurdie P.J., and Holmes, S. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8:4.

Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., and Tedersoo, L. 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47:D259-D264.

Oksanen J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., de Caceres, M., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C.J.F.,

and Weedon, J. 2022. vegan: community ecology package. R package Version 2.4-3. https://CRAN.R-project.org/package=vegan.

Omoboye, O.O., Oni, F.E., Batool, H., Yimer, H.Z., De Mot, R., and Höfte, M. 2019. Pseudomonas cyclic lipopeptides suppress the rice blast fungus *Magnaporthe oryzae* by induced resistance and direct antagonism. Frontiers in Plant Science 10:901.

Op De Beeck, M., Lievens, B., Busschaert, P., Declerck, S., Vangronsveld, J., and Colpaert, J.V. 2014. Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. PLoS ONE 9:e97629.

Paasch, B.C. and He, S.Y. 2021. Toward understanding microbiota homeostasis in the plant kingdom. PLoS Pathogens 17:e1009472.

Paulson, J.N., Stine, O.C., Bravo, H.C., and Pop, M. 2013. Differential abundance analysis for microbial marker-gene surveys. Nature Methods 10:1200-1202.

Perfect, S.E., and Green, J.R. 2001. Infection structures of biotrophic and hemibiotrophic fungal plant pathogens. Molecular Plant Pathology 2:101-108.

Qiu, Z., Verma, J.P., Liu, H., Wang, J., Batista, B.D., Kaur, S., de Araujo Pereira, A.P., Macdonald, C.A., Trivedi, P., Weaver, T., Conaty, W.C., Tissue, D.T., and Singh, B.K. 2022. Response of the plant core microbiome to *Fusarium oxysporium* infection and identification of the pathobiome. Environmental Microbiology 24:4652-4669.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41:D590-D596.

R Core Team. 2023. R: a language and environment for statistical computing. Vienna, Austria: R foundation for Statistical Computing. http://www.r-project.org.

Rodriguez, R. J., White Jr, J.F., Arnold, A.E., and Redman, R.S. 2009. Fungal endophytes: diversity and functional roles. New Phytologist 182:314-330.

Ryan, E.T. 2013. The intestinal pathobiome: its reality and consequences among infants and young children in resource-limited settings. The Journal of Infectious Diseases 208:1732-1733.

Seybold, H., Demetrowitsch, T.J., Hassani, M.A., Szymczak, S., Reim, E., Haueisen, J., Lübbers, L., Rühlemann, M., Franke, A., Schwarz, K., and Stukenbrock, E.H. 2020. A fungal pathogen induces systemic susceptibility and systemic shifts in wheat metabolome and microbiome composition. Nature Communications 11:1910.

Silby, M.W., Winstanley, C., Godfrey, S.A., Levy, S.B., and Jackson, R.W. 2011. Pseudomonas genomes: diverse and adaptable. FEMS Microbiology Reviews 35:652-680.

Skamnioti, P., and Gurr, S.J. 2009. Against the grain: safeguarding rice from rice blast disease. Trends in Biotechnology 27:141-150.

Song, C., Zhu, F., Carrión, V.J., and Cordovez, V. 2020. Beyond plant microbiome composition: exploiting microbial functions and plant traits via integrated approaches. Frontiers in Bioengineering and Biotechnology 8:896.

Spoel, S.H., Johnson, J.S., and Dong, X. 2007. Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proceedings of the National Academy of Sciences 104:18842-18847.

Sweet, M.J., and Bulling, M.T. 2017. On the importance of the microbiome and pathobiome in coral health and disease. Frontiers in Marine Science 4:9.

Tian, D., Chen, Z., Lin, Y., Liang, T., Chen, Z., Guo, X., Wang, F., and Wang, Z. 2021. The interaction between rice genotype and *Magnaporthe oryzae* Regulates the assembly of rice root-associated microbiota. Rice 14:40.

Vayssier-Taussat, M., Albina, E., Citti, C., Cosson, J.F., Jacques, M.A., Lebrun, M.H., Le Loir, Y., Ogliastro, M., Petit, M.A., Roumagnac, P., and Candresse, T. 2014. Shifting the paradigm from pathogens to pathobiome: new concepts in the light of meta-omics. Frontiers in Cellular and Infection Microbiology 4:29.

Wang, W., Zhai, Y., Cao, L., Tan, H., and Zhang, R. 2016. Endophytic bacterial

and fungal microbiota in sprouts, roots and stems of rice (*Oryza sativa L.*). Microbiological Research 188-189:1-8.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer, New York, USA.

Zeng, Q., Man, X., Huang, Z., Zhuang, L., Yang, H., and Sha, Y. 2023. Effects of rice blast biocontrol strain *Pseudomonas alcaliphila* Ej2 on the endophytic microbiome and proteome of rice under salt stress. Frontiers in Microbiology 14:1129614.

Zhang, J., and Luo, Y. 2017. Degree centrality, betweenness centrality, and closeness centralityin social network. Proceedings of the 2017 2nd International Conference on Modelling, Simulation and Applied Mathematics (MSAM2017) https://doi.org/10.2991/msam-17.2017.68.

Zhang, J., Cook, J., Nearing, J.T., Zhang, J., Raudonis, R., Glick, B.R., Langille, M.G., and Cheng, Z. 2021. Harnessing the plant microbiome to promote the growth of agricultural crops. Microbiological Research 245:126690.

면역 유전자 변이 품종과 벼도열병 감염으로 밝혀낸 벼의 패쏘바이옴

김 태 온

초 록

패쏘바이옴은 질병을 마이크로바이옴 단계에서 연구할 때 쓰이는 개념이다. 패쏘바이옴은 마이크로바이옴의 일부이며 병균이나 미생물불균형 현상과 연관되는 생물학적 환경을 지칭한다. 이 연구는 벼도열병에 관련된 패쏘바이옴 연구이다. 벼도열병은 벼에서 발생할 수 있는 가장 중요한 질병이며 쌀 생산량의 30%까지도 영향을 끼친다. 이 연구에서는 벼도열병 패쏘바이옴에 관련해서 두 가지 실험을 했다. 첫 실험은 유전자변이로 면역변이된 벼의 잎 내생균 패쏘바이옴을 관찰했다. 벼 품종 비교실험은 낙동과 유전자변이된 낙동인 *MoHTR1_OX* 에 행해졌으며 또한 동진과 유전자변이된 동진인 *OsMORE1a* 도 비교됐다. 둘째 실험은 벼도열병에 감염된 벼의 잎 내생균 패쏘바이옴을 관찰했다. 벼도열병에 감염되고 감염되지 않는 낙동 품종들을 비교했으며 동진에서도 동일하게 실험했다. 실험결과를 알파 다양성 분석기법인 Observed ASV, Shannon

index, 그리고 Simpson index 로 봤을 때 유전자변이나 벼도열병 감염은 벼 잎 내생균에 유의미한 차이를 만들지 못했다. 하지만 베타 다양성 분석 기법에서는 유전자변이나 벼도열병 감염이 벼 잎 내생균에 유의미한 차이를 만들었다. 미생물 구성 역시 속 단계에서 변화하였다. 이 연구의 결과물들은 추후 유전자변이와 질병 패쏘바이옴 내생균 연구에서 벼, 벼도열병, 그리고 내생균들간의 삼자관계를 파악하는 연구들에 도움이 될 수 있을 것이다.

주요어 : 패쏘바이옴, 벼도열병, 유전자 변이, 면역 변이, 벼

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