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Microbiome Responses to RYMV Infection: Insights from Rice Cultivation in Mali

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KAD and CC analyzed the results. Authors DD and KAD interpreted the results. Authors MW and DD supervised the work. Authors CC, SD and MW contributed to the final proof reading of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: The Rice Yellow Mottle Virus (RYMV) is one of the most significant viral pathogens affecting rice production in Sub-Saharan Africa, leading to yield losses as high as 100% in severely affected areas. In Mali, particularly in the Office du Niger region, RYMV poses a constant threat to rice cultivation. This study aimed to explore the microbiome diversity associated with RYMV-infected and non-infected rice plants, using next-generation sequencing (NGS) and metagenomics approaches. The presence of RYMV was confirmed by RT-PCR, and microbial DNA was extracted for sequencing.

Results: A total of 40 samples (leaves and roots) were collected from both infected and non-infected plants. The results revealed alterations in bacterial community composition between infected and non-infected plants. Alpha diversity indices, such as Shannon and Simpson indicated reduced microbial diversity in infected plants. Notably, certain bacterial genera, including Bacillus, Pseudomonas, and Kaistobacter, were statistically more abundant in non-infected plants, suggesting their potential role in conferring resistance to RYMV.

Conclusions: This study provided new insights into the microbial dynamics associated with RYMV infection and highlights the potential for leveraging the rice microbiome in developing biocontrol strategies to manage viral diseases. Future research should focus on isolating and characterizing the functional roles of these beneficial microorganisms in enhancing rice resistance to RYMV. That will serve for sustainable agriculture strategies development.

Keywords: Rice Yellow Mottle Virus (RYMV); metagenomics; bacterial microbiome.

ABBREVIATIONS

RYMV : Rice Yellow Mottle Virus
NGS : Next Generation Sequencing
RT-PCR : Reverse Transcription-Polymerase Chain Reaction

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial staple crop, essential for global food security, providing sustenance to more than half of the world's population. In Sub-Saharan Africa, demand for rice has seen a rapid increase, driven by population growth and dietary shifts (Seck et al., 2012). In Mali, rice cultivation is of significant economic importance, particularly in the Office du Niger region, which accounts for about 30.79% of the country's total cereal production (Doumbia et al., 2020). However, despite substantial local production, Mali remains reliant on rice imports to meet its growing food demands, underscoring the challenges faced in increasing domestic productivity.

One of the most severe threats to rice cultivation in West Africa is the Rice Yellow Mottle Virus (RYMV), which causes significant yield losses, ranging from 60% to 100% in severely affected areas (Sarra et al., 2009; Kouassi et al., 2005). RYMV is endemic to Africa and primarily affects irrigated rice fields, where it spreads rapidly through insect vectors and mechanical means (Pinel-Galzi et al., 2018). The virus, first identified in Kenya in 1966, has since spread to most rice-growing regions of Africa, including Mali, where it poses a persistent threat to rice production (Traoré et al., 2009; Sokpe Longue et al., 2021).

Although the impact of RYMV on rice yield has been well-documented, relatively few studies have focused on the interactions between the virus and the bacterial microbiome associated with infected and non-infected rice plants. Microorganisms, particularly endophytic and rhizospheric bacteria, play a critical role in plant health by enhancing resistance to pathogens, improving nutrient uptake, and inducing systemic resistance (Berendsen et al., 2012; Mendes et al., 2013). Understanding the microbial dynamics in RYMV-infected plants could provide valuable insights into the ecological and functional shifts that occur in response to viral infection and may open new avenues for developing biocontrol strategies.

Recent advances in metagenomics and deep sequencing technologies have enabled more comprehensive studies of plant-associated microbial communities (Raza & Wu, 2021). These technologies provide the tools to explore the genetic and functional diversity of microbial populations, offering insights into how viral infections alter microbial composition and how beneficial microbes can be harnessed to mitigate disease impact (Dongmo Nanfack et al., 2021).

In the context of RYMV, the application of these tools could lead to a better understanding of the microbial mechanisms that enhance or suppress viral infection, as demonstrated by studies on the rice microbiome (Hyun & Yong-Hwan Lee, 2018; Roman-Reyna et al., 2021).

Moreover, research on the biogeography of RYMV transmission has revealed that environmental factors, including microbial communities, play a significant role in the emergence and spread of the virus (Pinel-Galzi et al., 2018). In Mali, transmission has been linked to vector organisms, such as cows, donkeys, and grass rats, which facilitate the mechanical spread of RYMV in irrigated rice fields (Sarra & Peters, 2003). Given the increasing importance of integrated biocontrol strategies, understanding the role of microbial communities in viral resistance is critical.

Our previous study (Diallo et al., 2024) revealed significant changes in the microbial community structure of infected plants. This present work focused on in-depth genetic and functional analysis to better understand how bacterial diversity can influence rice plant resistance against the virus.

2. MATERIALS AND METHODS

Sampling site: Samples for this study were collected at the Office du Niger region in Mali, a key rice-growing area covering approximately 100,000 hectares (Fig. 1) (Doumbia et al., 2020). The region has a Sudano-Sahelian climate, characterized by a distinct rainy season (June to October) and a dry season. The soils in this area are clay-loam, providing optimal conditions for rice cultivation, and the extensive irrigation network makes it a prime rice production zone (Seck et al., 2012).

Rice plant sampling: A total of 40 samples (leaves and roots) were collected from both RYMV-infected and non-infected rice plants. The samples were obtained from five sampling points in fields infected. In total, 20 samples were collected from infected plants and twenty (20) samples from non-infected plants.

Infected plants were identified based on the presence of typical RYMV symptoms, such as yellow mottling of leaves and stunted growth. Non-infected plants were selected from fields without visible disease symptoms.

RNA Extraction and RT-PCR for viral detection: For the detection of Rice Yellow Mottle Virus (RYMV), total RNA was extracted from rice leaves and roots using the RNeasy Plant Mini Kit (QIAGEN), which is optimized for high-purity RNA isolation from plant tissues (Riley et al., 2014). The RNA was treated with DNase I to remove contaminating genomic DNA (Qi et al., 2011), ensuring the purity of the RNA for downstream analysis. The extracted RNA was used as a template for Reverse Transcription-PCR (RT-PCR) to confirm the presence of RYMV. Reverse transcription into complementary DNA (cDNA) was carried out using the SuperScript III First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. Specific primers, Prymv1 and Prymv2, targeting a conserved region of the viral genome were used to amplify the RNA, resulting in a 670 bp product in the infected samples (Pinel et al., 2000). Samples that showed no amplification were confirmed as virus-free. RT-PCR was chosen due to its sensitivity and specificity for viral detection in plant tissues (Koenig et al., 1997).

Microbial DNA extraction: Following RNA extraction for viral detection, microbial DNA was extracted from the same rice plant tissues (leaves and roots) using the PowerSoil® DNA Isolation Kit (QIAGEN). This kit is optimized for extracting DNA from soil and plant samples while minimizing inhibitors that can affect PCR reactions (Miller et al., 1999). The extracted DNA represented a mixture of microbial communities associated with the plant, including bacteria and fungi.

DNA library preparation: The extracted microbial DNA was processed to prepare DNA libraries using the Nextera XT DNA Library Prep Kit (Illumina Inc.), which fragments the DNA to the appropriate size for sequencing and adds index adapters for sample identification during next-generation sequencing (NGS) (Baym et al., 2015).

Next-generation sequencing: Next-generation sequencing (NGS) was performed using the Illumina MiSeq platform. The 16S rRNA gene was amplified for the identification of bacterial communities. The MiSeq platform was selected due to its high accuracy and throughput, making it ideal for detailed microbial community profiling (Caporaso et al., 2012).

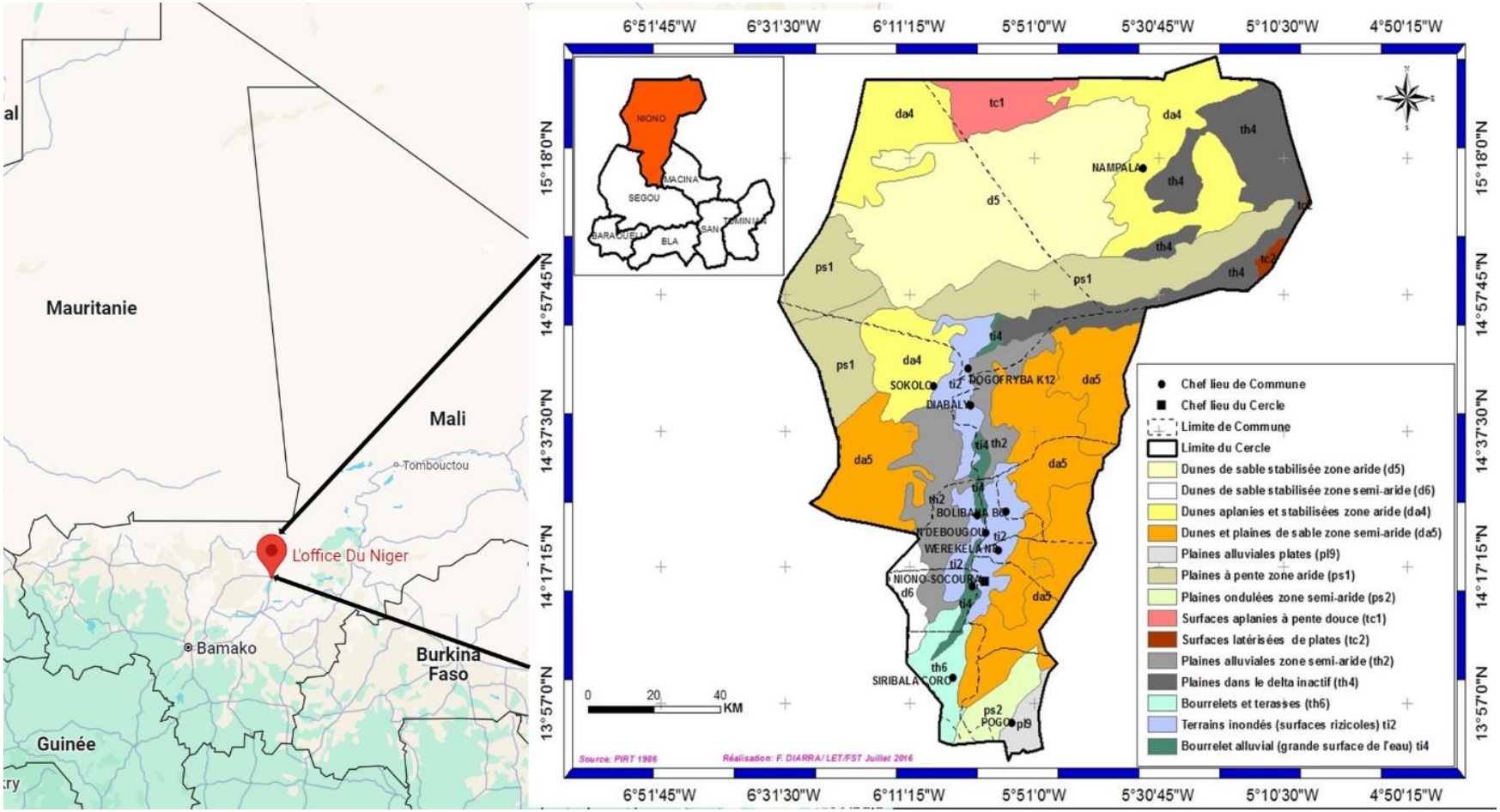

Fig. 1. Map of study site (Office du Niger)

Metagenomics analysis: The resulting sequence data were processed using QIIME 2 (Quantitative Insights Into Microbial Ecology) software, which is designed for high-throughput microbial ecology data analysis (Bolyen et al., 2019). The bacterial 16S rRNA sequences were compared against the Greengenes database for taxonomic identification.

Diversity analysis: To measure species richness and evenness, the Shannon and Simpson diversity indices were calculated. The Shannon Index accounts for species richness and evenness; higher values indicate greater diversity. The Simpson Index reflects the probability that two randomly selected individuals belong to the same species; it is inversely proportional to diversity. Beta diversity was assessed using Principal Component Analysis (PCA), which visualizes differences in microbial community structure between groups.

Statistical analysis: Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted to assess statistical differences in microbial community composition between infected and non-infected samples (Anderson, 2017). PCA plots were used to visualize the variation in microbial communities across the sample groups.

3. RESULTS

3.1 Molecular Confirmation of Rice Yellow Mottle Virus in Infected Rice Plants

The RT-PCR results confirmed the presence of Rice Yellow Mottle Virus (RYMV) in 17 samples of infected plants. The use of the specific primers Prymv1 and Prymv2 revealed the presence of amplicons approximately 670 bp in length in the suspected samples, while the samples from non-infected plants showed no amplification, thus confirming the absence of the virus.

3.2 Genetic Diversity of Bacterial Microbiome Associated with Rice Plants

All samples were sequenced using Illumina platform at University of Minnesota, USA. The analysis of 16S rRNA sequences identified a total of 42 bacterial phyla in both infected and non-infected rice plants. Three *phyla* predominated across all samples: Proteobacteria (58% of the total relative abundance), Firmicutes (19.4%), and Bacteroidetes (5.2%). A total of 94 classes, 193 orders, 308 families, and 614 genera were identified among the bacteria present in the samples (Fig. 2).

At the genus level, the most abundant genera were Bacillus, Thiobacillus, Pedomicrobium, and Kaistobacter (formerly classified under Sphingomonas), along with 47 other genera. Differences in microbial composition between infected and non-infected plants.

The results of the analysis of variance (ANOVA) indicated no significant difference ($p > 0.05$) between the microbial communities of infected and non-infected plants, meaning the observed variations do not support rejecting the null hypothesis.

3.3 Alpha and Beta Diversity of Microbial Communities

The alpha diversity indices, measured using Simpson and Shannon indices, revealed that non-infected plants exhibited slightly higher microbial diversity compared to infected plants. The Simpson index ranged between 0.6439 and 0.9667, indicating relatively high evenness in both groups, while the Shannon index varied between 1.5324 and 3.1727, with higher values observed in non-infected plants (Fig. 3).

Fig. 3 shows these measurements in boxplot form to visualize the dispersion of Shannon and Simpson index values between samples. The boxplots show the variability in microbial diversity between infected and uninfected plants (Diallo et al., 2024).

Beta diversity analyses based on Principal Component Analysis (PCA) plots showed the formation of two distinct groups, corresponding to infected and non-infected plants. The first two principal components explained 14.2% and 12.09% of the total variation in microbial communities, respectively. Although the number of identified microorganisms did not show drastic differences, the clear separation of the groups indicates that viral infection leads to differences in the composition of microbial communities, as reflected in the overall structure of the microbial communities (Fig. 4).

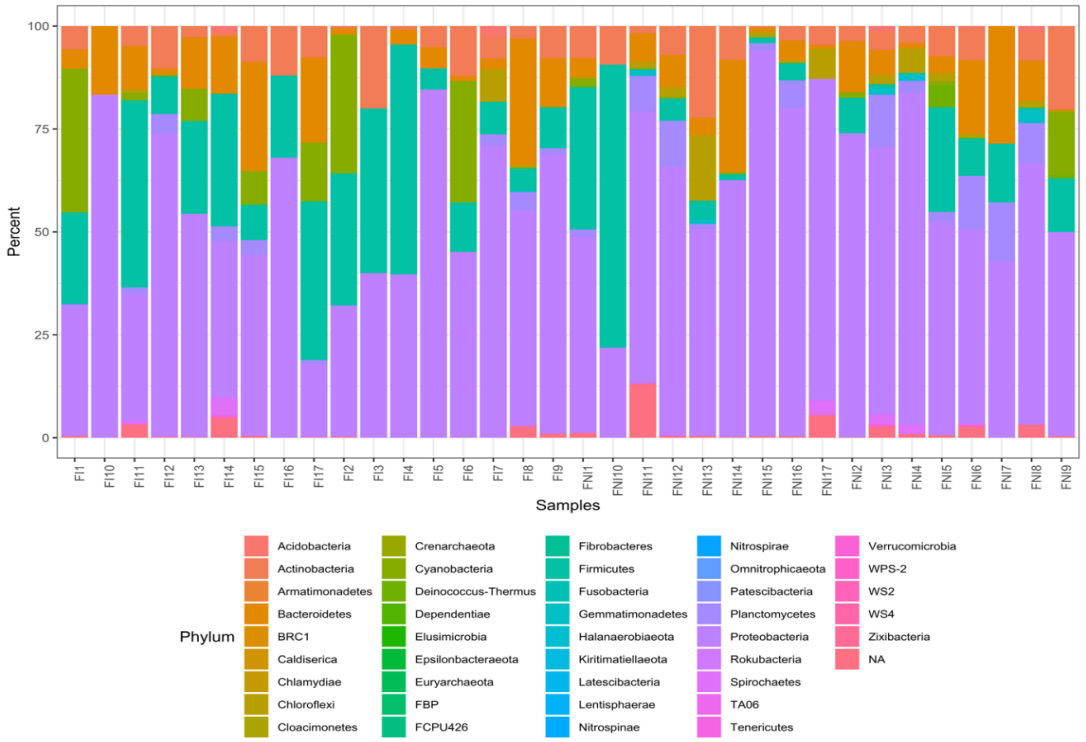

Fig. 2. Relative abundance of different *phyla* in the microbial communities from infected plant samples (FI1 to FI17) and non-infected plant samples (FNI1 to FNI17) (Diallo et al., 2024)

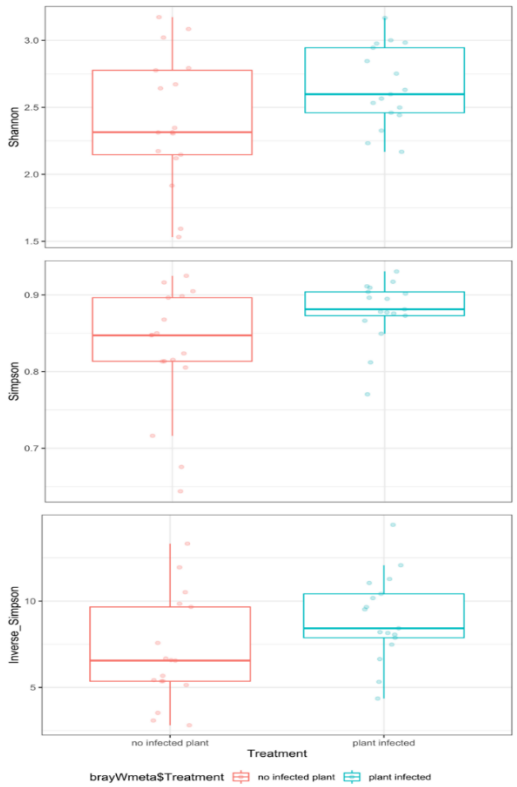

Fig. 3. Measurements in boxplot form to visualize the dispersion of Shannon and Simpson index values between samples

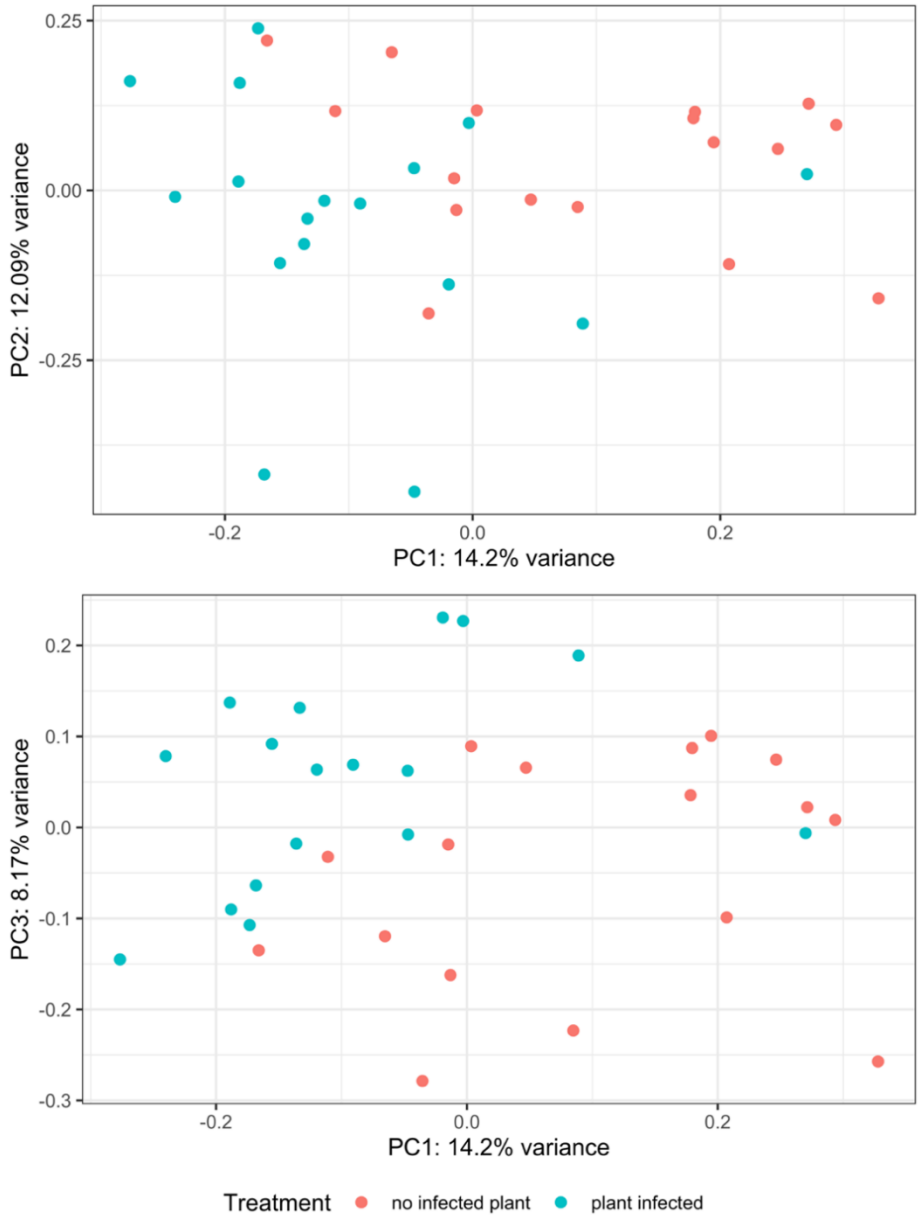

Fig. 4. Principal Component Analysis (PCA) of the overall microbial community in infected and non-infected plants (Diallo et al., 2024)

Identification of bacteria of interest: Although the relative abundance of microorganisms did not vary significantly between infected and non-infected samples, certain bacterial genera, such as Bacillus, Pedomicrobium, and Thiobacillus, were more abundant in non-infected plants. These genera are known for their role in inducing systemic acquired resistance (SAR) mechanisms and enhancing the plant's immune defenses (Pieterse et al., 2014). Additionally, the increased presence of certain microorganisms in non-infected plants suggests that they may play a protective role against viral infection.

4. DISCUSSION

The results of this study revealed significant differences in microbial community composition between RYMV-infected and non-infected rice plants, highlighting the profound impact viral infections can have on the bacterial microbiome. The observed changes align with findings from recent research, which shows that viral infections can disrupt the natural balance of microbial communities, leading to a reduction in beneficial taxa and an increase in opportunistic pathogens.

4.1 Genetic Diversity of Bacterial Microbiome in Infected vs. Non-Infected Plants

The alpha diversity results showed a slight reduction in microbial diversity in RYMV-infected plants, as measured by the Shannon and Simpson indices. This reduction is consistent with prior studies on viral diseases in plants, such as the study by Dongmo Nanfack et al. (2021), which highlighted changes in the rice seed microbiome in response to abnormal sprouting, indicating that stress conditions such as viral infections can reduce microbial diversity and alter community composition. Additionally, Pinel-Galzi et al. (2018), in their exploration of the biogeography of viral emergence, specifically in the case of RYMV, suggested that viral infection can lead to shifts in the microbial community structure, affecting both the richness and functional potential of associated microbiota.

The observed beta diversity analysis, visualized through PCA plots, further supports this conclusion by showing distinct community structures between infected and non-infected plants. This mirrors findings by Hyun and Yong-Hwan Lee (2018) in their characterization of the rice microbiome, where viral infections were found to significantly impact microbial composition, particularly in disrupting beneficial symbiotic relationships within the holobiome. Similarly, the study by Roman-Reyna et al. (2021) emphasized the role of whole-genome sequencing in uncovering the changes in leaf microbiomes in response to external stressors, such as viral infections, demonstrating that rice microbiomes are highly sensitive to biotic stresses.

The hypothesis that RYMV infection alters the bacterial microbiome by modifying the recruitment of beneficial microorganisms is strongly supported by evidence from metagenomic studies. The work of Raza and Wu (2021), for example, emphasizes the use of deep sequencing and metagenomics to detect subtle changes in microbial community structure caused by viral diseases. Their research indicates that viral infections can significantly alter the functional potential of the microbiome by suppressing beneficial taxa and promoting the emergence of opportunistic microorganisms.

RYMV infection also appears to have affected the genetic structure of microbial communities. While the relative abundance of dominant phyla remained stable, the infection induced subtle changes in the composition of Operational Taxonomic Units (OTUs). These changes could reflect a reduction in genetic variability and the resilience of microbial communities, making infected plants more vulnerable to biotic and abiotic stresses (Shade et al., 2012). The results of the PCA analyses suggest that viral infection may restructure microbial interactions within infected plants, thereby reducing the ability of these communities to support the plants' defense mechanisms.

4.2 Role of bacteria in Protection Against the Virus

Several bacterial genera identified in this study, including Bacillus, Thiobacillus, Pedomicrobium, and Kaistobacter, were more abundant in non-infected plants, suggesting a protective role against RYMV. Bacillus spp. have been widely studied for their ability to enhance plant defense mechanisms through induced systemic resistance (ISR), as noted by Pieterse et al. (2014). The antiviral properties of Bacillus, such as the production of lipopeptides like surfactin and fengycin, which directly inhibit viral replication, have been well-documented, supporting the hypothesis that these bacteria play a key role in viral suppression. This mirrors findings in the study by Sokpe Longue et al. (2021), where Central African rice accessions with higher resistance to RYMV were found to have a more robust microbiome, particularly dominated by beneficial microbes like Bacillus.

Furthermore, Kaistobacter and Pseudomonas, both identified in higher abundance in non-infected plants, are known for their roles in improving plant immunity and growth. Research by Innerebner et al. (2011) highlights that Kaistobacter can produce metabolites that activate plant immune responses, while Pseudomonas spp. are known to promote induced systemic resistance (ISR) through jasmonic acid (JA) and ethylene signaling pathways. These microbes likely contribute to the enhanced resistance observed in non-infected plants by promoting a stronger immune response, a mechanism also suggested by Mendes et al. (2011) and Berendsen et al. (2012).

The bacteria identified in non-infected plants could contribute to resistance against RYMV by modulating the plant’s immune responses through the production of secondary metabolites

and antiviral compounds. For example, lipopeptides produced by Bacillus are well-documented for their ability to directly inhibit pathogens and induce defense responses in plants (Bakker et al., 2018). It is likely that these microorganisms play a role in regulating the defense mechanisms of non-infected plants, helping them better resist the virus.

This hypothesis of microbial involvement in viral resistance is further supported by studies on viral transmission in rice crops. For instance, Sarra and Peters (2003) found that viral vectors like grass rats and other herbivores can influence the spread of RYMV, but microbial communities in the plant can mediate the severity of infection, indicating that the microbiome plays a protective role by mitigating the effects of viral infection.

4.3 Potential for Biocontrol Strategies

The identification of beneficial microbes in non-infected plants opens avenues for developing biocontrol strategies against RYMV. Bacillus spp., with their known ability to induce systemic acquired resistance (SAR), have been proposed as candidates for microbial inoculants in rice fields. Similar strategies have been successful in controlling bacterial and viral diseases in rice, as demonstrated by Sanogo et al. (2019), who used Bacillus-based bioformulations to control bacterial blight in rice crops. The combination of genetic resistance and microbial inoculants, as proposed by Pinel-Galzi et al. (2018), could be a promising integrated approach for sustainable RYMV management.

Additionally, the use of metagenomics and functional profiling, as discussed by Raza and Wu (2021), could aid in the identification of microbial genes that contribute to viral suppression. Field trials are necessary to evaluate the efficacy of these microbes in different agroecological conditions, including the potential influence of viral vectors like grass rats, as explored by Sarra and Peters (2003). Combining biocontrol agents with an understanding of the viral ecology and transmission dynamics, as suggested by Pinel-Galzi et al. (2018), could offer a comprehensive solution for controlling RYMV in rice cultivation systems.

5. CONCLUSION

This study demonstrates that RYMV infection significantly alters the bacterial diversity and structure in rice plants, reducing beneficial microbial taxa and promoting opportunistic pathogens. Non-infected plants exhibit higher microbial diversity, with Bacillus, Kaistobacter, and Pseudomonas playing potential roles in enhancing resistance against RYMV. These findings suggest that leveraging beneficial microorganisms could provide a biocontrol strategy to manage viral infections in rice cultivation, reducing reliance on chemical pesticides. Further research is needed to explore the mechanisms by which these microbes enhance viral resistance in rice and to use microbes for biocontrol trials and functional metagenomics.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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