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Quality Evaluation of Cambodian Rice Seeds Under Organic Tillage and Green Manure Production Systems

Singy Ho[1], Malyna Suong[1,2*], Kakada Oeum[1], Vichny Vicheth[1], Rada Kong[3,4], Florent Tivet[3,4], Myriam Adam[5,6], Lionel Moulin[1,7]

[1] *Research and Innovation Center, Institute of Technology of Cambodia, Russian Federation Blvd., Phnom Penh, Cambodia*
[2] *International Relations Office, Institute of Technology of Cambodia, Russian Federation Blvd., Phnom Penh, Cambodia*
[3] *Cambodian Conservation Agriculture Research for Development Centre, Department of Agricultural Land Resources Management, General Directorate of Agriculture, MAFF, Phnom Penh, Cambodia*
[4] *CIRAD, UPR AIDA, University of Montpellier, Montpellier, France*
[5] *CIRAD, UMR AGAP Institut, F-34398 Montpellier, France*
[6] *Faculty of Agriculture and Food Processing, National University of Battambang, Battambang, Cambodia*
[7] *Plant Health Institute of Montpellier (PHIM), IRD, CIRAD, INRAE, Institut Agro, Univ Montpellier, Montpellier, France*

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Abstract: *Rice is a staple crop in Asia. In Cambodia, agroecological practices were introduced in Preah Vihear Province, testing organic systems with conventional tillage and no agrochemical inputs (CT) and cover cropping as green manure (GM) between rice cycles to enhance soil fertility and yield. This study compared the yield and quality of six Cambodian rice varieties (Phka Rumduol and the other five local cultivars) across 17 biplot systems (CT/GM). We assessed yield, physical quality (biomass, brokenness, moisture), nutritional quality (amylose and protein content) using NIR, and microbiological quality (seed-borne phytopathogens and mycotoxin-producing fungi) using PCR and amplicon sequencing. The results showed that GM practices significantly increased yield (12–19%) for all varieties except Phka Rumduol. Physical traits (biomass, brokenness, moisture) and amylose content of the nutritional traits differed between varieties but not between practices. Protein content varied both by varietal and practice effects: Neang Om had the highest protein (8.72 ± 0.6%), while CT seeds averaged 3% higher protein than GM. Diagnostic PCR detected common phytopathogens* (C. oryzae, Pantoea spp., X. oryzae, B. glumae*) and mycotoxin-producing fungi* (Alternaria spp.), *with differences across varieties but no consistent effect of practice. Amplicon sequencing of Phka Rumduol revealed additional phytopathogens and greater seed microbiome diversity in GM compared to CT. Overall, GM practices demonstrated strong potential to enhance yield and promote microbial diversity while maintaining seed quality consistent with market requirements. Meanwhile, CT systems preserved slightly higher protein levels. Long-term trials are needed before recommending wide-scale adoption to policymakers and farmers.*

Keywords: Rice crop; Agricultural practices; Seed quality; Near Infrared Spectroscopy; Phytopathogen

1. INTRODUCTION

Rice (*Oryza sativa*) is an important food crop in the world, with nearly 40% of the world's population consuming rice as their main staple food. Asia accounts for about 86% of the world's rice area and contributes about 90% of global rice production [1]. In Cambodia, around 85% of rural residents work as rice farmers [2]. The farmers produce the long-cycle fragrant indica Phka rumduol variety for export during the rainy season, while short-cycle indica varieties are used for local or neighboring countries' markets at the end of the rainy season [3]. Farmers use mainly conventional practices, which rely on soil tillage and high agro-chemical inputs (fertilizers, pesticides), leading to a decrease in soil fertility and

* *Correspondence: malyna.suong@itc.edu.kh*

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environmental pollution. Fertilisers and pesticides are used by the majority of holdings in agriculture and only 9% (174,000 holdings) do not use chemical inputs [4].

Achieving sustainable rice production requires overcoming many challenges, including climate change, access to arable land, pressure from pests and diseases, and limited water resources. Many phytopathogens reduce rice yield [5], and several fungi reduce rice quality by producing mycotoxins. For several years, agroecology practices have been introduced, for example, in Preah Vihear in organic production systems, with farmers using conventional tillage and no chemical inputs (CT practice) or by growing cover crops between rice cycles that are incorporated by tillage into soil as green manure to improve soil fertility and rice yield [6].

Various options are available for improving the quality, safety, and nutritional value of rice: biofortification, improving the yield of high-quality traditional varieties through breeding, improvements in milling technologies, the promotion of healthy rice products, as well as agronomic practices, such as organic or conservation agriculture that improve soil fertility and rice yields [7].

In order to promote sustainable rice production, it is important to assess the quality of rice seeds produced in agroecological production systems and evaluate their suitability for the market. Rice quality relies on physical [8], nutritional (amylose, protein) [9], and microbiological (presence of seedborne phytopathogens and mycotoxin-producing fungi) [10] properties.

This study aimed to compare the quality of rice seeds from six local varieties of rice, the most commonly grown by local farmers in the study area, using CT and GM organic farming methods in Preah Vihear province. We analyzed physical properties (size, weight, breakage), nutrition (amylose and protein content) and the presence of seed-borne phytopathogens and mycotoxin-producing fungi by molecular methods (diagnostic PCR and amplicon barcoding sequencing).

2. METHODOLOGY

2.1 Rice fields and seed sample collection

The seed samples were harvested between October and November 2023 (depending on rice cycle duration) from rice fields biplots located at GPS coordinates 13°25'00.1"N 105°08'42.3"E, close to Boh village, Rik Reay commune, Rovieng district, Preah Vihear province, Cambodia. Seventeen rice fields were cultivated by 17 farmers under supervision by GDA-DALRM and CIRAD agronomists, as part of the ASSET project (AFD-EU). Each rice field included six rice varieties (**Table 1**) grown in biplot systems with two organic agricultural practices: conventional practice (CT) with the use of tillage and without any inputs (no fertilizers and no pesticides) and green manure (GM) with cover crop between rice cycles incorporated in soil by tillage, and no inputs. Around 100 g of seed samples per variety/rice field/practice were dried, then kept at room temperature until further analysis.

2.2 Seed sample preparation

One hundred seeds were counted and weighed using a precision balance to assess the average seed mass. To dehusk seeds, we used an Automatic Rice Husker TR-260 (Kett Electric Laboratory Co. Ltd), followed by additional manual separation for seeds that did not dehusk well. After dehusking, the seeds were stored in ziplock bags and stored at room temperature until the next step.

Table 1. Rice varieties and their characteristics

Rice Variety	Cycle	Characteristics	Market
Kraches	medium	Long slender grain, soft texture [11]	Local
Neang Om	medium	Slightly sweet taste, dry texture	Local
Neang Ork	medium	Long slender grain, medium texture [11]	Local
Neang Sar	short	Bold grain, hard texture [11]	Local
Prich	medium	Medium grain, low shattering	Local
Phka Rumduol	long	Extra-long grain, soft texture, distinct aromatic flavor	Highest value; best-selling, export

2.3 Analysis of seed breakage

To evaluate seed brokenness, 5 g of dehusked seeds were separated manually on an A4 green plastic sheet, and pictures were captured and then analyzed for the number and size of full and broken seeds using ImageJ (version Java 1.8.0_345). After adjusting the scale, seeds were detected by adjusting the color threshold, and the particle analysis function was used to calculate the size and number of full and broken seeds.

2.4 Analysis of moisture content and nutritional value of seeds using Near InfraRed Spectroscopy

The moisture, protein, and amylose content from the dehusked rice seeds was evaluated using a Near Infrared Rice Composition Analyser AN-920 (Kett Electric Laboratory Co. Ltd.) calibrated for long rice composition, following the manufacturer's instructions. Means of three replicates were used for each sample. Phka Rumduol cultivar was used for calibration.

2.5 DNA extraction of rice seeds

In order to recover DNA from seeds for the detection of phytopathogens by PCR, we incubated 10 seeds per sample in a lysis buffer composed of 2M KOH and 60% PEG200, then 1 µl of the lysate was used as template for PCR [12].

For amplicon barcode sequence analysis of seed surface microbiota, five replicates of 10 rice seeds for each condition were put in 1.5 ml Eppendorf and 1 ml of sterile deionized water was added; then the tubes were incubated in a bath sonicator for 10 min at maximum sonication input. Seeds were then removed from tubes, and tubes were centrifuged at 6000 g for 10 mins. Supernatant was removed and the pellet was resuspended in 800 µl of lysis buffer from the DNeasy PowerSoil Pro Kits (Qiagen), then transferred to Powersoil tubes and DNA extracted following kit protocol [13].

2.6 Molecular detection of phytopathogens in rice seeds

Polymerase Chain Reaction (PCR) was used to detect the presence of DNA of rice pathogens and toxin-producing fungi in 6 varieties grown in 5 biplot systems. Each PCR reaction contained 14.875 µl of sterile pure water, 5 µl of PCR buffer 5X (Go-Taq, Promega), 2 µl of dNTP mix (10 mM each), 1 µl of each primer (forward and reverse, at 10 uM), and 0.125 µl of GoTaq DNA Polymerase (5U/µl). 1 µl of DNA was added per reaction. A negative control with no DNA was always added to evaluate contamination by environmental DNA. A positive control was also added that contains primers and a known DNA targeted by the primers, to evaluate PCR mix performance. All reactions were amplified in a PCR thermocycler (Labnet International, Inc.) with its specific program depending on primers for the targeted pathogen (**Table 2**).

2.7 Gel electrophoresis of PCR products

PCR products were visualized by gel electrophoresis of a 1.5% agarose gel in Tris-Acetate-EDTA (TAE) 1X buffer (Sigma-Aldrich). Then, 10 µl was loaded in each well, and the electrophoresis was run at 80 volts for 50 mins. A ladder (SmartLadder, Eurogentec) was included to assess PCR band sizes. After migration, the gel was incubated in 50 ml of water with 5 µl of 10,000X Gelgreen fluorescent dye for 10 min, then rinsed in water for 15 min and viewed and pictured in a Bluebox Transilluminator (minipcr©).

Table 2. PCR primers and programs used to detect phytopathogens and mycotoxin-producing fungi

Primer name	Sequence (5'-3')	Target	Size (bp)	PCR program*	References
Xo3756F Xo3756R	CATCGTTAGGACTGCCAGAAG GTGAGAACCACCGCCATCT	*Xanthomonas oryzae* (hyp. Protein)	331	35 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 45 s).	[14]
toxB_F toxB_Rd	GCATTTGAAACCGAGATGGT TCGCATGCAGATAACCRAAG	*Burkholderia glumae* (*toxB*)	508	35 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 45 s).	[14]
Dir1ITSAlt Inv1ITSAlt	TGTCTTTTGCGTACTTCTTGTTTCCT CGACTTGTGCTGCGCTC	*Alternaria species* (ITS)	370	35 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 45 s).	[15]
CercoGpdFor CercoGpdRev	TGTCTTYACCACYACCGA TGGSACACGCATRGACAT	*Cercospora spp.* (*gdph*)	411	35 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 45 s).	L. Moulin, unpublished
PAN_KK263F PAN_KK263R	GCGAGCCAATCGACATTA CGAGTAACCTGAGTGTTCAG	*Pantoea spp.* (*atpD*)	263	35 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 45 s)	[16]

Note: All PCR programs had an initial step at 94°C for 2 min, and a final extension step for 10 min at 72°C

2.8 Amplicon barcode sequencing

In order to capture the diversity of bacteria and fungi on rice seeds in Phka Rumduol variety, we produced 16S and ITS amplicon sequences using amplicon barcode sequencing methodology. It consists of first a PCR using universal primers to amplify taxonomic markers: the 16S rRNA V3V4 variable region for bacteria and the intergene sequence 2 (ITS2) for microeukaryotes (including fungi); then the PCR products are used to build libraries, a barcode is added to each sample library, and all libraries are then mixed and sequenced on an Illumina sequencing machine (MiSeq). DNA quality control, amplicon PCR (16S V3V4 and ITS2), library preparation (using the Nextera XT Index V2 kit) and sequencing on an Illumina MiSeq (2 x 300 bp, paired-end) were done by Macrogen company. The 16S V3V4 region was amplified with primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC), while ITS2 was amplified with ITS3F (GCATCGATGAAGAACGCAGC) and ITS4R (TCCTCCGCTTATTGATATGC) [17]. A no-template control was included. Sequencing yielded around 50,000 reads for 16S and ITS.

2.9 Statistical analysis

R version 4.4.0 was used for the statistical analyses of phenotypic data, using the packages rstatix, ggplot2, and multcompView. As all of our data did not follow a normal distribution (*p-value* or p <5% with the Shapiro-Wilk test), non-parametric Kruskal-Wallis tests (α=5%) followed by

pairwise Wilcoxon tests (p.adjust.method = "bonferroni") were used to assess the significance of differences between means. A letter code was used to represent the different statistical groups in the boxplot figures. Box plots with identical letters are not statistically different, while box plots with no letters in common are statistically different at α=5%.

2.10 Microbiome analysis of amplicon sequences

FastQ files (16S, ITS) were processed independently in FROGS pipeline [18] under the Galaxy environment [19] using the usegalaxy.fr server (Institut Français de Bioinformatique). Sequences per sample were first curated from primers and assembled, then a clustering swarm algorithm was used (using an aggregation distance of 1), chimeric sequences were removed, and OTUs were affiliated at the taxonomic level using BLAST on Silva 138.2 (for bacteria) and UNITE 8.2 (for eukaryotes) databases. Taxonomic binning and differential analysis (Wilcoxon tests) were done with NAMCO [20].

3. RESULTS AND DISCUSSION

This study evaluated the seed quality of six Cambodian varieties cultivated in 17 biplot systems under green manure (GM) and conventional tillage (CT) organic production in Preah Vihear Province of Cambodia. The assessment covered yield performance and key quality parameters, including physical, nutritional, and microbiological properties of the seeds.

3.1 Yield of six rice varieties grown under CT and GM organic practice

The yield of six rice varieties grown in 17 biplots under CT or GM treatment is shown in **Fig 1**.

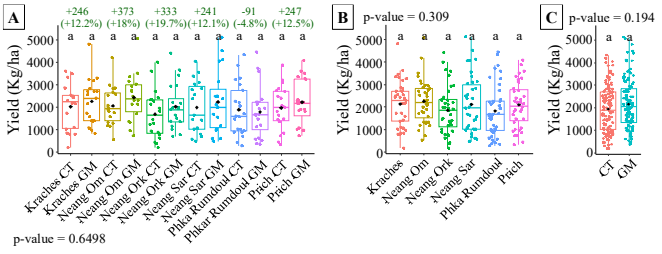

Fig. 1. Comparison of the yield of 6 rice varieties produced in CT and GM biplots. A: by variety and practice, B: by variety, C: by practice. Letters above each box plot represent statistical groups (Wilcoxon test). Numbers in green in A is the increase of yield between CT and GM (net increase (%))

Globally, there were no significant differences in yield between treatments when comparing variety, practices, or their combination, meaning the variance was too high between plots to obtain statistically significant results. According to **Fig 1**, the yield of rice was increased by 12.1-19.7% in GM compared to CT. Five out of six rice varieties produced in GM gave higher yield in mean and median than CT. In contrast, only Phka rumduol varieties produce a yield in GM, 4.8% lower than CT. Despite statistically non-significant differences, the tendency is thus for an increase in GM compared to CT, but variability in farmer plots' history and use of land, and habits, may have generated high variability in yield within treatments. Green manure can improve soil fertility and increase rice yields by enhancing the availability of NPK in the soil and, consequently, nitrogen absorption [21]. It is a described strategy for increasing rice grain yield in agroecosystems [22], but applying excessive amounts of green manure provides no additional benefits and can even have a negative impact on rice production and soil health [23].

3.2 Assessment of physical quality of seeds in six rice varieties cultivated in CT and GM systems

The physical quality of the rice seed was determined by the biomass of 100 rice seeds and the brokenness of the rice seed. In **Fig. 2**, we show the results of biomass of 100 seeds as boxplots organized by varieties and agricultural practice (A), by variety only (B), and by practice (C). The weight of 100 seeds is a proxy for the average biomass of seeds.

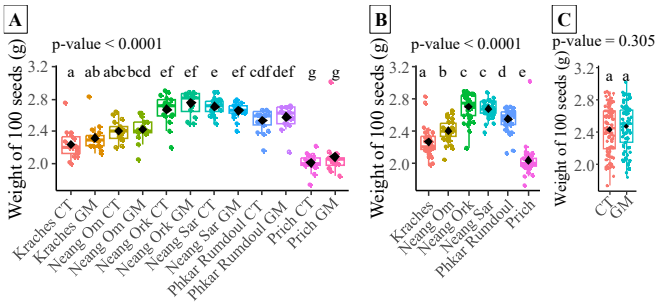

Fig. 2. Comparison of the biomass of 100 seeds of 6 rice varieties produced in 17 CT and GM biplots. With A: by variety and practice, B: by variety only, C: by practice only (CT/GM). The p-value above each plot is the result of a Kruskal-Wallis test. The letters above each box plot represent the statistical groups from pairwise Wilcoxon tests.

The average biomass of 100 seeds was significantly different between varieties (**Fig. 2.B**) but not by practice and variety (**Fig. 2.A**) or by practice only (**Fig. 2.C**). Neang Ork produced the heaviest weight (2.71 ± 0.132 g), and Prich produced the lightest weight (2.04 ± 0.199 g). These differences can be explained by their different genetic background [24] and the intrinsic characteristics of each variety [25].

The second physical parameter that we investigated was the breakage of seeds following dehusking. We plotted the %

of broken seeds as boxplots in **Fig. 3**. As shown in **Fig. 3.A**, several varieties differ in their % of broken seeds (p = 0.017), but there was no significant difference between the CT and GM practices (**Fig. 3.B)** or the combination practice-variety (**Fig. 3.C**). The rice variety Neang Om was the least broken out of the six varieties, with a breakage rate between 14% and 28%.

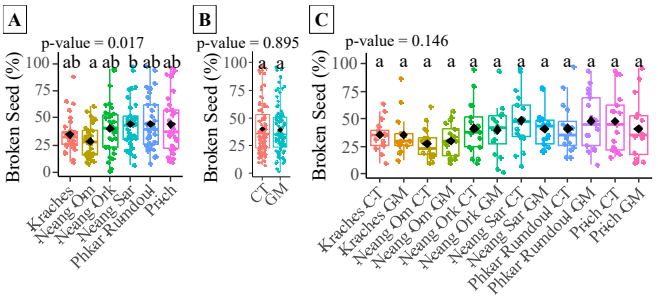

Fig. 3. Comparison of seed brokenness of rice varieties produced in CT and GM biplots, between varieties (A), agricultural practice (B), and varieties + practice (C). Same legend for statistics as in **Fig. 1**.

The broken rice rate is a critical metric that exerts a considerable influence on the appearance, processing, and economic value of rice [26]. A multitude of factors, including hormones, enzymes, nutrients, and environmental influences, have been demonstrated to play a role in the incidence of seed brokenness [27]. Furthermore, additional factors that have the potential to compromise the integrity of rice include the timing of planting, water management practices, soil nitrogen levels, the cultivar of rice, the time of harvest, the handling of paddy, and the moisture content of the grain [28].

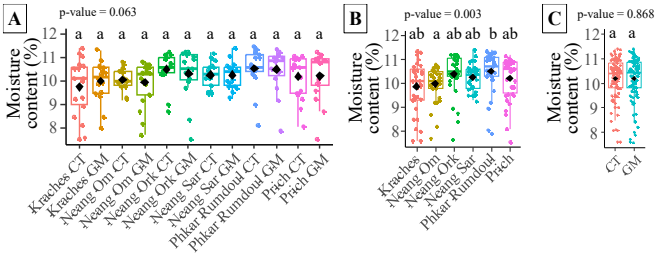

Fig. 4. Comparison of moisture content of rice varieties produced in CT and GM biplots, by variety and agricultural practice (A); by variety (B) and practice (C). Same legend for statistics as in **Fig. 1**.

Results for moisture content of seeds is shown in **Fig. 4**. Significant differences were only detected between varieties (p = 0.003) (**Fig. 4.B**), but not in practice-variety (**Fig. 4A**) or practice only (**Fig. 4C**). Grain is typically harvested with a moisture content of 18–25 percent on a wet basis; however, this can vary significantly according to factors such as the stage of maturation, the season, weather conditions and drying facilities [29]. In order to maintain rice quality and enable long-term preservation, grain must be dried to a moisture content of 11–14 percent [30]. Rice seeds with a moisture level of around 6% can be stored for an extended period [31].

3.3 Assessment of seed nutritional quality in rice varieties grown under CT and GM

The nutritional quality of rice seeds was assessed by evaluating the protein and amylose content with an NIRS precalibrated for long rice. The protein and amylose contents are presented as boxplots in **Fig. 5**. The average protein content of rice seeds was 8.2 ± 0.8 %. As shown in **Fig. 5A**, the boxplots of variety in CT or GM share a common letter code corresponding to a statistical group, meaning they are not statistically different. When all varieties were mixed to analyze the global effect of the practice (**Fig. 5C**), the seed protein content was significantly higher (p = 0.007) in CT (8.36%) compared to GM (8.09%), with a 3.3% increase. Significant differences (p < 0.01) in protein content were also observed between varieties (**Fig. 5.B**). The rice variety that contained the highest protein content was Neang Om with 8.72 ± 0.6 % and the lowest was Prich with 7.47 ± 0.8 %. Usually, the protein content of rice ranges from 7-10% [28], which is in the range of our measurements. The differences observed between varieties are a known feature [9]. Rice's protein levels have a significant impact on its nutritional value and processing capabilities [8].

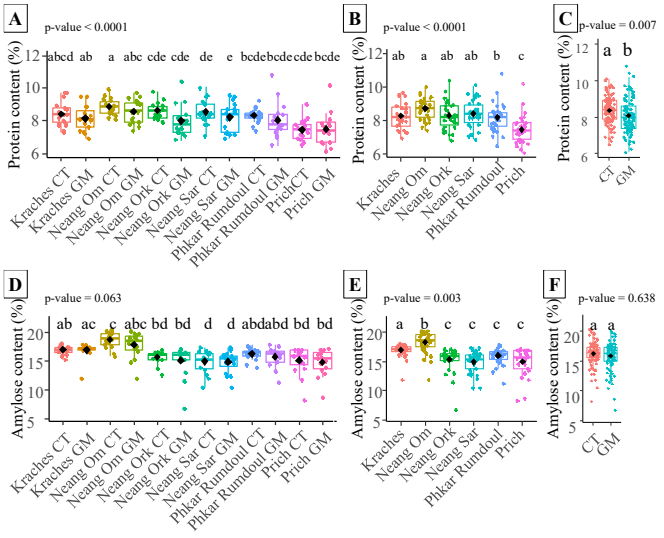

Fig. 5. Protein and amylose content in rice varieties produced in CT and GM. A,B,C: protein content (%) in samples by variety and agricultural practice (A); by variety (B) and practice (C), D, E, and F: amylose content by variety and agricultural practice (D) and by variety (E) and practice (F). Same legend for statistics as in **Fig. 1**.

The average amylose content of rice seeds is shown in **Fig. 5.D, E** and **F**, with an average of 16.0 ± 2.1 %. A significant difference (p = 0.003) was observed between varieties (**Fig. 5.E**), but not in the practice-variety combination (**Fig. 5.D**) or practice only (**Fig. 5.F**). Neang Om had the highest amylose content (18.1 ± 1.8 %) while Prich had the lowest (15.1 ± 2.3 %). The amylose content of rice varieties can be classified as waxy (0–2%), very low (5–12%), low (12–20%), intermediate (20–25%), or high (25– 33%) [9]. Here, all our varieties can be considered as low, which offers a good cooking quality with soft and sticky texture, slow digestibility, and reduced sugar release [32].

The statistical results are summarized in the **Table 3**.

Table 3. Sum-up of statistical analyses of the impact of variety and practice on rice quality parameters.

Rice seed	Variety	Practice (GM/CT)
Biomass	***	ns
Breakage	*	ns
Moisture	*	ns
Protein	***	**
Amylose	***	ns

Note: ns (not significant): $p > 0.05$, *: $p < 0.05$, ** : $p < 0.01$, *** : $p<0.001$

3.4. Detection of seed-borne microorganisms

To evaluate the presence of seed-borne phytopathogens, we focused on frequent seed-borne bacterial rice pathogens, *Cercospora oryzae* (Narrow brown spot), *Pantoea spp.* and *Xanthomonas oryzae* (leaf blight), and *Burkholderia glumae* (panicle blight), and on fungal *Alternaria* species, which produce various toxic compounds, including mycotoxins in rice [33]. PCR was used with specific primers for each microbial target (see methods), on 10 seeds for six varieties in 5 biplot systems, and the detection results are given as a percentage of positive PCR signals in **Fig. 6**.

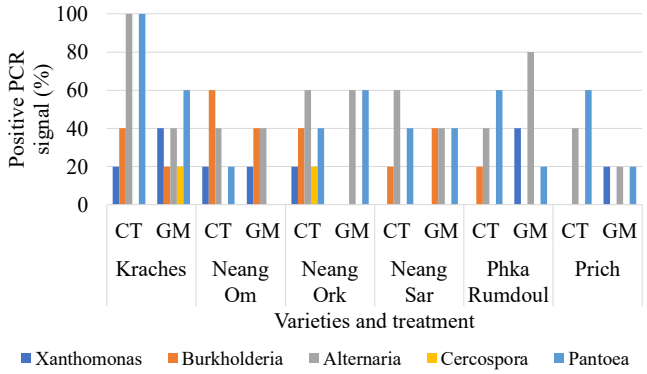

Fig. 6. Bar plot of positive PCR signal for the detection of the seed-borne phytopathogens *C. oryzae*, *Pantoea spp.*, *X. oryzae*, *B. glumae,* and mycotoxin producer *Alternaria* species.

As a result, *Cercospora oryzae* was detected only in Kraches-GM and Neang Ork-CT with very low frequency (10% of the sample). *Xanthomonas oryzae* was detected in five varieties (no detection in Neang Sar) and at low frequency (0-30% of samples). It was detected on Phka rumduol and Prich in GM biplots but not in their corresponding CT system. *Burkholderia glumae* was also detected in five varieties (not in Prich) in 0-50% of the samples, and on Phka rumduol in CT but not GM. *Pantoea* was detected in all varieties (10%-80% of the sample) and treatments (100% in Kraches-CT, to 20% in Neang Om-CT, Phka rumduol-GM and Prich-GM), except Neang Om grown under GM practice. *Alternaria sp.* was detected in all varieties with some variation (30% in Prich and 70% in Kraches) and practices (100% in Kraches-CT, to 20% in Prich-GM), with no clear impact of variety or practice on its frequency.

In order to better describe the presence of pathogens in rice seeds, an amplicon sequencing strategy was used. This method allows for the detection of all microorganisms present in a given sample by sequencing taxonomic markers (16S for bacteria, ITS for fungi). In **Fig. 7**, the relative abundance of the top 15 species of bacteria **(Fig. 7.A)** and fungi **(Fig. 7. B)** detected from rice seeds of Phka Rumdoulvariety grown under CT and GM. In amplicon sequences, the expected pathogens already detected by PCR (*B. glumae, Pantoea dispersa, Cercospora oryzae, Alternaria padwickii*) were detected, but also new phytopathogens, such as *Bipolaris oryzae* (brown spot disease), *Nigrospora oryzae* (leaf spot disease), and *Curvularia lunata* (Brown leaf spot). We could not detect *Xanthomonas oryzae* while it had a low signal by PCR, but other *Xanthomonas* species (*X. translucens, X. sacchari*) were detected.

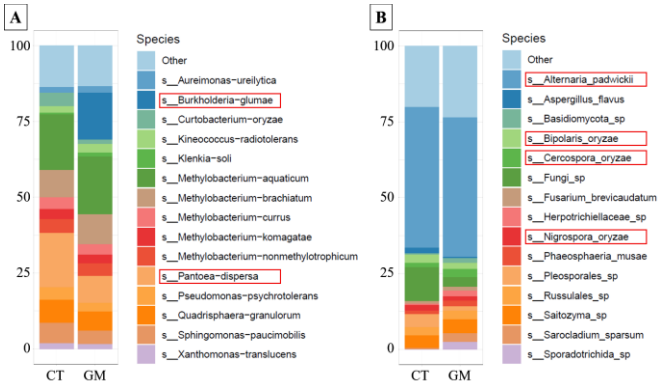

Fig. 7. Top 15 species detected in amplicon sequences of 16S of bacteria (A) and ITS of fungi (B) from rice seeds of Phka rumduol varieties produced in CT and GM. Known rice phytopathogens are surrounded in red.

X. oryzae was found by PCR only in a few samples, so our investigation on the DNA of 5 replicates on 10 seeds might have missed it. Microorganisms known to be beneficial for plants, including *Methylobacterium aquaticum*, *M.*

brachiatum, and *Sphingomonas paucimobilis* [31, 32, 33] were also detected. Beta-diversity analysis showed significant differences in the Phka rumduol seeds microbiome between GM and CT (ITS and 16S Permanova test $p = 0.013$, $p = 0.044$, respectively), while alpha diversity indices showed a tendency for an increase in GM compared to CT, but this was not significant. A differential analysis test on microbial taxa abundances (Wilcoxon test on log-centered normalized abundances) was performed to detect differences at the species level between CT and GM. There was a total of six bacteria and 16 fungi differentially abundant between practices, of which only one is a known phytopathogen, *Pantoea dispersa* ($p = 0.032$), more abundant in CT.

The impact of fertilizer and green manure is known to impact the structure of microbial communities, plant development, and soil carbon sequestration [37]. As observed with our data, microbial communities of seeds can be greatly impacted by sustainable agriculture methods, which can result in structural and compositional changes as well as the promotion of pertinent agroecosystem activities [38].

4. CONCLUSIONS

We compared different seed quality criteria in two organic practices performed by farmers on 17 biplots in Preah Vihear. Our analysis was performed on seeds produced in 2023, which was the second year of practice of CT and GM on these biplots. We did not find a strong impact of the practice on the quality of seeds from the 6 varieties investigated, but a tendency to a yield increase was detected. The values of biomass, breakage and nutrient contents measured were in the range of acceptability for the market. The biodiversity of the seed microbiome was also different between GM and CT, with a tendency for increased biodiversity in GM. Further work is required to measure the impact of green manure after more years of practice, as significant changes are often described in the literature at later times, usually at least 4 to 5 years [39]. In conclusion, the incorporation of green manure in organic rice production shows strong potential to enhance yield and promote biodiversity, while maintaining seed quality consistent with market standards. However, these findings require confirmation through longer-term field evaluation.

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REFERENCES

[1] FAO, *A regional rice strategy for sustainable food security in Asia and the Pacific*. 2014.

[2] M. Neang, P. Méral, O. Aznar, and C. Déprés, "NeangFinalVersion (1)," *Int. J. Agric. Resour. Gov. Ecol.*, vol. 13, no. 4, pp. 1–19, 2017, doi: 10.1504/IJARGE.2017.088402.

[3] Cambodia Rice Federation, "5th World Best Rice Award," Cambodia Rice Federation. Accessed: Jun. 24, 2024. [Online]. Available: http://www.crf.org.kh

[4] NIS, MoP, MAFF, and FAO, "Cambodia Agriculture Survey 2022: Production Method and the Environment," 2022.

[5] S. Savary, L. Willocquet, S. J. Pethybridge, P. Esker, N. Mcroberts, and A. Nelson, "The global burden of pathogens and pests on major food crops," *Nat. Ecol. Evol.*, vol. 3, no. 3, pp. 430–439, 2019, doi: 10.1038/s41559-018-0793-y.

[6] FAO, MAFF, and CASIC, *Bottom-up solutions to promote conservation agriculture in Cambodia*. Rome, Italy: FAO, 2022. doi: 10.4060/cc2698en.

[7] P. Nickens, D. R. Ader, M. Miller, P. Srean, T. Gill, and S. Huot, "Conservation agriculture and cover crop adoption by smallholder farmers in Cambodia: Understanding perceptions, challenges, and opportunities for soil improvement," *Adv. Agric. Dev.*, vol. 4, no. 4, pp. 39–48, 2023, doi: 10.37433/aad.v4i4.387.

[8] R. C. Bautista and P. A. Counce, "An Overview of Rice and Rice Quality," *Cereal Foods World*, vol. 65, no. 5, p. 9, 2020, doi: 10.1094/cfw-65-5-0052.

[9] A. A. Abera, E. E. Tadesse, B. B. Abera, and N. Satheesh, "Effect of rice variety and location on nutritional composition, physicochemical, cooking and functional properties of newly released upland rice varieties in Ethiopia," *Cogent Food Agric.*, vol. 7, no. 1, pp. 1–23, 2021, doi: 10.1080/23311932.2021.1945281.

[10] S. M. Ebrahim Zainali, "Evaluation Seed-born Fungi of Rice [Oryza sativa L.] and that Effect on Seed Quality," *J. Plant Pathol. Microbiol.*, vol. 05, no. 04, 2014, doi: 10.4172/2157-7471.1000239.

[11] O. Makara, P. Sovuthy, C. Sareth, O. Bunna, and U. Sokco, "The Stung Chinit Irrigation and Rural Infrastructure Project (SCIRIP) CHAMBAK," Kampong Thom, Cambodia, 2004. [Online]. Available: https://elibrary.maff.gov.kh/download/book/64d447bb37d5d

[12] F. Zhang *et al.*, "Rapid detection of Fusarium fujikuroi in rice seeds and soaking water samples based on

recombinase polymerase amplification-lateral flow dipstick," *Plant Pathol.*, vol. 73, no. 3, pp. 590–601, 2024, doi: 10.1111/ppa.13831.

[13] Qiagen, "Quick-Start Protocol DNeasy® PowerSoil® Pro Kit," no. May. QIAGEN®, p. https://www.qiagen.com/us/resources/resourcedetail, 2019. [Online].

[14] J. M. Lang *et al.*, "Genomics-based diagnostic marker development for Xanthomonas oryzae pv. oryzae and X. oryzae pv. oryzicola," *Plant Dis.*, vol. 94, no. 3, pp. 311–319, 2010, doi: 10.1094/PDIS-94-3-0311.

[15] M. Á. Pavón, I. González, M. Rojas, N. Pegels, R. Martín, and T. García, "PCR detection of alternaria spp. in processed foods, based on the internal transcribed spacer genetic marker," *J. Food Prot.*, vol. 74, no. 2, pp. 240–247, 2011, doi: 10.4315/0362-028X.JFP-10-110.

[16] M. Bangratz *et al.*, "Design of a new multiplex PCR assay for rice pathogenic bacteria detection and its application to infer disease incidence and detect co-infection in rice fields in Burkina Faso," *PLoS One*, vol. 15, no. 4, pp. 1–13, Apr. 2020, doi: 10.1371/journal.pone.0232115.

[17] K. Oeum *et al.*, "Comparison of plant microbiota in diseased and healthy rice reveals methylobacteria as health signatures with biocontrol capabilities," *Front. Plant Sci.*, no. October, pp. 1–19, 2024, doi: 10.3389/fpls.2024.1468192.

[18] F. Escudié *et al.*, "FROGS: Find, Rapidly, OTUs with Galaxy Solution," *Bioinformatics*, vol. 34, no. 8, pp. 1287–1294, 2018, doi: 10.1093/bioinformatics/btx791.

[19] The Galaxy Community, "The Galaxy platform for accessible , reproducible, and collaborative data analyses : 2024 update," *Nucleic Acids Res.*, vol. 52, no. Web Server issue, pp. 83–94, 2024, doi: https://doi.org/10.1093/nar/gkae410.

[20] A. Dietrich *et al.*, "Namco: a microbiome explorer," *Microb. Genomics*, vol. 8, no. 8, pp. 1–16, 2022, doi: 10.1099/mgen.0.000852.

[21] S. Marahatta, "Nutrient Management Practice for Conservation and Conventional Agriculture Practices on Rice Based System at Central Terai of Nepal," *Agric. Dev. J.*, no. July, pp. 1–13, 2022, doi: 10.3126/adj.v16i1.51616.

[22] M. R. Alizadeh, A. Dabaghi, and F. R. Ajdadi, "Effect of final paddy moisture content on breaking force and milling propertiesof rice varieties Drought stress effects of engineering and milling properties of rice View project," *Elixir Agric.*, vol. 36, no. May 2014, pp. 3186–3189, 2011, [Online]. Available: www.elixirjournal.org

[23] Q. Yue *et al.*, "Rotation with green manure increased rice yield and soil carbon in paddies from Yangtze River valley, China," *BENEFITS PADDY RICE-GREEN MANURE Rotat. Rotat.*, vol. 33, no. 4, pp. 589–599, 2023, doi: 10.1016/j.pedsph.2022.11.009.

[24] P. E. R. Prahardini, E. Fidiyawati, S. S. Antarlina, and T. Sudaryono, "Characteristic of grain and rice quality from five superior varieties support food sustainability," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 1253, no. 1, pp. 1–8, 2023, doi: 10.1088/1755-1315/1253/1/012016.

[25] R. T. Adhiguna, S. Sutrisno, and R. Thahir, "Comparison of physical properties, proximate composition and milling quality of rice grains from different branches within a panicle," *Int. J. Sci. Eng. Res.*, vol. 7, no. 9, pp. 1647–1652, 2016, [Online].

[26] J. Ye, Z. Hu, Y. Chen, D. Fu, and J. Zhang, "Identification of broken rice rate based on grading and morphological classification," *Lwt*, vol. 215, no. 2025, pp. 1–7, 2025, doi: 10.1016/j.lwt.2024.117175.

[27] H. Wu, Q. He, and Q. Wang, "Advances in Rice Seed Shattering," *Int. J. Mol. Sci.*, vol. 24, no. 10, pp. 1–17, 2023, doi: 10.3390/ijms24108889.

[28] O. Da, "Factors affecting cracking and breakage of rice (Oryza sativa L.) during milling: A Review," *J. Food Technol. Preserv.*, vol. 3, no. 1, pp. 1–4, 2021, doi: 10.36266/JFTP/117.

[29] FAO, "Grain crop drying, handling and storage," in *Rural structures in the tropics: Design and development*, O. A. Januarius, O. G. Lawrence, C. M. Geoffrey, and H. J. Chepete, Eds., Rome, Italy: Food and Agriculture Organization, 2011, pp. 363–386. [Online]. Available: https://www.fao.org/3/i2433e/i2433e.pdf

[30] N. A. Mohd Ramli *et al.*, "A New Method of Rice Moisture Content Determination Using Voxel Weighting-Based from Radio Tomography Images," *Sensors (Basel).*, vol. 21, no. 11, pp. 1–19, 2021, doi: 10.3390/s21113686.

[31] B. Lu, J. Sun, N. Yang, X. Wu, X. Zhou, and J. Shen, "Quantitative detection of moisture content in rice seeds based on hyperspectral technique," *J. Food Process Eng.*, vol. 41, no. 8, pp. 1–7, 2018, doi: 10.1111/jfpe.12916.

[32] P. Srinang, S. Khotasena, J. Sanitchon, S. Chankaew, S. Jogloy, and T. Monkham, "New Source of Rice with a Low Amylose Content and Slow In Vitro Digestion for Improved Health Benefits," *Agronomy*, vol. 13, no. 2622, pp. 1–12, 2023, doi: 10.3390/agronomy13102622.

[33] C. Schwarz, C. Tiessen, M. Kreutzer, T. Stark, T. Hofmann, and D. Marko, "Characterization of a genotoxic impact compound in Alternaria alternata infested rice as Altertoxin II.," *Arch. Toxicol.*, vol. 86, no. 12, pp. 1911–1925, Dec. 2012, doi: 10.1007/s00204-012-0958-4.

[34] S. Dutta, S. Y. Choi, and Y. H. Lee, "Temporal Dynamics of Endogenous Bacterial Composition in Rice Seeds During Maturation and Storage, and Spatial Dynamics of the Bacteria During Seedling Growth," *Front. Microbiol.*, vol. 13, no. July, pp. 1–15, 2022, doi: 10.3389/fmicb.2022.877781.

[35] C. Schrader, A. Schielke, L. Ellerbroek, and R. Johne, "PCR inhibitors - occurrence, properties and removal," *J. Appl. Microbiol.*, vol. 113, no. 5, pp. 1014–1026, 2012, doi: 10.1111/j.1365-2672.2012.05384.x.

[36] S. Yang *et al.*, "Growth-promoting sphingomonas paucimobilis ZJSH1 associated with Dendrobium officinale through phytohormone production and nitrogen fixation," *Microb. Biotechnol.*, vol. 7, no. 6, pp. 611–620, 2014, doi: 10.1111/1751-7915.12148.

[37] J. Xu, L. Si, X. Zhang, K. Cao, and J. Wang, "Various green manure-fertilizer combinations affect the soil microbial community and function in immature red soil," *Front. Microbiol.*, vol. 14, no. December, pp. 1–16, 2023, doi: 10.3389/fmicb.2023.1255056.

[38] P. Mondaca *et al.*, "Effects of sustainable agricultural practices on soil microbial diversity, composition, and functions," *Agric. Ecosyst. Environ.*, vol. 370, no. 6373, pp. 1–12, 2024, doi: 10.1016/j.agee.2024.109053.

[39] S. Yadav *et al.*, "Effect of Organic and Inorganic Nutrient Sources on Productivity, Grain Quality of Rice and Soil Health in North-West IGP: A Review," *Int. J. Curr. Microbiol. Appl. Sci.*, vol. 8, no. 12, pp. 2488–2514, 2019, doi: https://doi.org/10.20546/ijcmas.2019.812.293