A comparison of the arbuscular mycorrhizal fungal communities among Bangladeshi modern high yielding and traditional rice varieties

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Acknowledgements

We are grateful to all farmers to allow sampling in their rice fields. We also thank Kasper Van Acker and Gerrit Peeters for their assistance in the laboratory.

Abstract

Aims

Arbuscular Mycorrhizal Fungi (AMF) are ubiquitous soil microorganisms playing a vital role in the functioning of agricultural ecosystems. Although AMF are generally considered to have a low host specificity, it has been suggested that modern plant breeding has selected crop genotypes that are more selectively associated with AMF, possibly resulting in modern high yielding varieties (HYV) having a lower AMF diversity than traditional crop varieties. Whether this is true for paddy rice varieties under field conditions is not known so far. Here, we aimed at comparing differences of AMF communities among modern HYV and traditional rice varieties.

Methods

We collected root and soil samples of five Bangladeshi rice varieties (two traditional and three modern HYV) from 40 different rice fields and quantified AMF richness, diversity and community composition through high throughput amplicon sequencing of the small subunit (SSU) of the ribosomal RNA cistron.

Results

Overall, 75 AMF OTUs, distributed over six AMF families with Glomeraceae as predominant family were found. After accounting for differences in soil conditions, we found that AMF diversity significantly differed among the five varieties and was higher in the traditional than modern varieties. The composition and structure of the AMF communities were distinct between the traditional and modern varieties. An indicator species analysis detected 9 OTUs significantly associated with traditional rice varieties, whereas no indicator OTUs were found for the modern HYV.

Conclusions

We conclude that modern breeding coupled with high fertilizer application rates provide a plausible explanation for the reduced AMF diversity and the different AMF community composition between Bangladeshi modern HYV and traditional varieties.

Keywords

Arbuscular mycorrhizal fungi (Glomeromycotina), Traditional varieties, Modern high yielding varieties (HYV), Host specificity, AMF richness and diversity, Crop plant breeding

Introduction

Arbuscular Mycorrhizal Fungi (AMF) are ubiquitous soil microorganisms known to play a key role in the functioning of natural and agricultural ecosystems (Verbruggen and Kiers, 2010; Genre et al., 2020). It is currently accepted that AMF have co-evolved with their hosts since plants conquered the terrestrial environment during the Ordovician Period more than 430 million years ago (Redecker et al., 2000; Strullu-Derrien et al., 2018). This extremely long-term co-evolutionary history suggests that AMF have facilitated the colonization of land by plants (Redecker et al., 2000). AMF belong to the subphylum Glomeromycotina and form a symbiosis with the roots of c. 72% of all land plants, acting as a living interface between plant roots and soil (Brundrett and Tedersoo, 2018). AMF associations are reported from all terrestrial ecosystems, including tropical to temperate forests, alpine, sand dunes, deserts, grassland, aquatic plants and agroecosystems. They are characterized by the formation of unique tree-shaped subcellular structures known as arbuscles within the cells of the plant root, which are considered to be the functional site for nutrient exchange (Parniske, 2008). Across the intimate cellular association between the two symbiotic partners, AMF supply mineral nutrients, especially phosphorus (P) and nitrogen (N), and receive carbon from their host plants, which usually increases plant growth (Smith and Read, 2008). AMF also protect the host plant against pathogens (Veresoglou and Rillig, 2012) and increases their tolerance against biotic (Harrier and Watson, 2004) and abiotic stress (Lenoir et al., 2016; Bahadur et al., 2019). In addition, they form an extensive mycelium hyphal network into the surrounding soil, improving soil structure and aggregation (Wilson et al., 2009).

Applying AMF inocula has become an appealing tool for sustainable agriculture due to the positive attributes of the mycorrhizal symbiosis (Berruti et al., 2016; Basu et al., 2018). Previous studies have shown benefits of AMF inoculation for various crops (Zhang et al., 2019) such as wheat (*Triticum aestivum*, Talaat and Shawky, 2012), maize (*Zea mays*, Estrada et al., 2013), rice (*Oryza sativa* L., Parvin et al., 2020), potato (*Solanum tuberosum*, Hijri, 2016) and tomato (*Solanum lycopersicum* L., Abdel Latef and Chaoxing, 2011), either under controlled or field conditions. Yet, despite the importance of AMF, their diversity and abundance in agricultural soils tend to be low compared to more natural ecosystems (e.g., Verbruggen et al., 2010), which has mainly been attributed to increased soil nutrient levels (Van Geel et al., 2017). Yet, AMF colonization rates do not only vary with changing abiotic soil conditions,

they are also dependent on the host plant species and even on the host plant genotype. For instance, plants from members of the genera *Amaranthus*, *Brassica* and *Urtica* are non-mycorrhizal while the genera *Bromus*, *Sorghastrum*, *Glycine* and *Zea* are moderate to good AMF host plants (with a colonization percentage ranging from 9 to 70%) (Wilson and Hartnett, 1998; Tawaraya, 2003; Miransari, 2012). Host genotype differences in response to AMF inoculation have been shown for tomato (*Solanum lycopersicum*, Calvo-Polanco et al., 2016), pepper (*Capsicum annuum*, Duc et al., 2017), wheat (*Triticum aestivum*, Hetrick et al., 1993), maize (*Zea mays*, An et al., 2010) and some other crops (Estaún et al., 2010). For example, for chickpea (*Cicer arietinum*), only 3 out of 13 cultivars responded positively to AMF inoculation (Bazghaleh et al., 2018). Also geographically different accessions of barrel clover (*Medicago truncatula*) and subterranean clover (*Trifolium subterraneum*) were shown to differ in growth and AMF colonization in response to inoculation (Ryan et al., 2016; Watts-Williams et al., 2018).

Although the precise genetic and biochemical drivers of the variation in AMF colonization rates and host plant response among different crop genotypes are still poorly known, it has been suggested that modern crop breeding might have negatively affected the capacity to establish AMF symbioses (Martín-Robles et al., 2018; Sawers et al., 2018; Martín-Robles et al., 2020). Since breeding programs are generally aimed at maximizing crop yield in high-input production systems, it is likely that a breeding process accompanied with high rates of fertilization has selected crop genotypes that are less responsive to the AMF symbiosis. For example, Hetrick et al. (1993) and Zhu et al. (2001) evaluated 26 wheat varieties and reported that modern varieties exhibited lower AMF colonization than old varieties. Turrini et al. (2016) also found substantial variability in AMF colonization between domesticated and wild genotypes of sunflower (*Helianthus annuus*). Yet, other studies did not find reduced AMF colonization in modern varieties, such as for oats (*Avena sativa*) and tomato (*Solanum lycopersicum*) when compared to wild (Koide et al., 1988; Bryla and Koide, 1990). How crop breeding has affected the ability to establish AMF symbioses has not been assessed for rice (*Oryza sativa* L.) so far.

Rice is one of the most important staple and forage crops in many regions of the world accounting for a production of 496.1 million metric tons in 2019 worldwide (FAOSTAT). *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice) are two distinct types of domesticated rice. The Asian rice varieties were domesticated from *Oryza rufipogon* and the African rice varieties were domesticated from *Oryza barthii* (Sweeney and McCouch, 2007). Although the symbiotic relationship between rice and AMF has been a topic of debate (Ilag et al., 1987; Vallino et al., 2009), several

studies in the field (Watanarojanaporn et al., 2013; Wang et al., 2015; Xu et al., 2017; Bernaola et al., 2018) and glasshouse (Solaiman and Hirata, 1998; Zhang et al., 2005, 2017) have shown that roots of both upland and lowland rice are colonized by AMF. Like in many other crops, high yielding varieties (HYV) have been developed to improve rice yields. These varieties require the application of fertilizers and pesticides to attain their full yield potential, often resulting in environmental pollution. Due to the multi-functional benefits of AMF to plant growth, AMF inoculation may have the potential to contribute to a more sustainable rice production (Porcel et al., 2016; Panneerselvam et al., 2017; Parvin et al., 2020). How AMF colonization and community composition vary across different rice varieties under field conditions, and especially how they differ among HYV and traditional varieties is yet not known. We thus sampled 40 Bangladeshi rice fields planted with three modern HYV (developed by the Bangladesh Rice Research Institute) and two traditional varieties. Our objectives were to: (i) assess differences in AMF diversity, community composition and structure in the roots among these five rice varieties by using high throughput amplicon sequencing; and (ii) to elucidate whether modern HYVs associated with different AMF than traditional varieties.

Material and Methods

Study area and sampling

We selected five different rice varieties grown in the northern part of Bangladesh (Dinajpur district). BR28, BR29 and BR58 are modern high yielding varieties, cultivated since 1994 (BR28, BR29) and 2012 (BR58). Shampakatar and Ushapari (also known as Pari) are local traditional rice varieties (cultivated by farmers in the Dinajpur district already for a long time). Each rice variety was collected from eight rice fields separated by a minimum distance of 2 km, totaling in 40 sampled rice fields (Fig. 1 and Table S1). Root samples were taken from five randomly selected rice plants within each field. We also took soil samples near each root sample to a depth of 0-20 cm for chemical analysis. In total, we collected 200 rice root and soil samples across the 40 rice fields.

Soil chemical analyses

Five grams of soil were weighed and suspended in deionized water in a ratio of 1:10 soil/water. The suspension was then shaken for 10 min at 650 rpm and pH was measured using a pH electrode. The Olsen P extraction method was used to extract plant available phosphate (Olsen, 1954). Two g of fresh soil were mixed with 25 mL of 0.5 M sodium bicarbonate and shaken for 30 min. The solution was then centrifuged for 5 min at 3500 g, followed by colorimetric analysis of the extract with an Evolution 201 UV-visible Spectrophotometer (Thermo Scientific, Waltham, MA, USA)

using the molybdenum blue method (Robertson et al., 1999). Moisture content was determined in 10 g of fresh soil as weight loss after drying the soil in an oven at 105 °C for 1 day. Organic matter was quantified as weight loss of 10 g of dry soil by combustion at 700 °C.

DNA extraction, PCR amplification and sequencing

The rice roots were rinsed with water to remove remaining soil particles and cut into small pieces (< 1 cm). Genomic DNA was extracted from 0.25 g of root material with the soil DNA Isolation Plus Kit (Norgen Biotek Corp., Thorold, ON, Canada) following the manufacturer's protocol. For PCR the DNA extracts were diluted 10 times. A 350 bp fragment of the small subunit (SSU) of the ribosomal RNA (rRNA) cistron was PCR amplified with the glomeromycotan-specific primers AMV4.5NF (5'- AAGCTCGTAGTTGAATTTTCG -3') and AMDGR (5'- CCCAACTATCCCTATTAATCAT -3') (Sato et al., 2005; Van Geel et al., 2014) tagged with sample-specific identifiers for dual-index sequencing (Kozich et al., 2013). PCR reactions were performed in a volume of 25 µL, containing genomic DNA (1 µL), dNTPs (1 µL), 10 µM primer (1 µL of each primer), 1X Titanium Taq PCR buffer (4 µL), Titanium Taq DNA polymerase (0.25 µL) (Clontech Laboratories, Palo Alto, CA, USA) and nuclease-free water (16.75 µL). The thermal cycling program consisted of an initial denaturation at 94 °C for 2 min, followed by 35 amplification cycles of denaturation at 94 °C for 20 s, annealing at 66 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were purified using the Agencourt AMPure XP kit (Beckman Coulter Life Sciences, Indianapolis, IN, USA) before quantification using the Qubit dsDNA HS Assay Kit and Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). The amplicons were pooled in equimolar concentrations, and the pooled amplicon library was loaded on an agarose gel, from where the amplicons of the expected size were excised and purified, using the QIAquick Gel Extraction Kit (Qiagen, Hamburg, Germany). After dilution to 2 nM, the amplicons were sequenced at the Genomics Core UZ Leuven on an Illumina Miseq platform using 2 x 250 paired end Illumina chemistry with v2 500 cycle reagent kit (Illumina, San Diego, CA, USA).

Bioinformatics

Sequences were obtained as de-multiplexed FASTQ files, which were processed and quality filtered using USEARCH (Edgar, 2013). In a first step, the *fastq_mergepairs* command was used to join the paired end reads on the overlapping region to form consensus sequences, which resulted mean merged read length with 219 bp. *fastq_filter* command was used to quality filter the reads with a maximum expected error of 1% for the individual sequences. Then using

fastq_uniques command, the sequences were dereplicated and sorted by abundance, and sequences that occurred only once in the entire dataset were excluded to improve the diversity estimates (Brown et al., 2015). Next, the sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using *cluster_otus* command. Chimeric reads of abundant reads were also discarded in this step. Finally, an OTU table was created with the *otutab* command using the UPARSE algorithm implemented in USEARCH. By performing a BLAST search against GenBank of the representative sequences, OTUs were assigned to AMF taxa. Taxonomic assignments were considered reliable when the BLAST score values were ≥ 300 . OTUs not belonging to the Glomeromycota or having a BLAST score < 300 were discarded. Finally, the representative sequence of each OTU was compared against the MaarjAM database (Opik et al., 2010). Only sequences with 95%-100% coverage and *E*- values close to zero were retained in the dataset. Samples covered by < 10 reads were omitted, which resulted in a final dataset of 144 symbiotic AMF communities of roots.

Statistical analysis

To prevent bias due to different sequencing depth, it is common to use rarefaction techniques to interpolate to a smaller number of sequences. The disadvantage of rarefaction is that point comparisons force to rarefy all samples down to the smallest sample size in the data set. As some of our samples contained a low number of sequences, we chose to use non-parametric asymptotic extrapolations to retrieve reliable and comparable measures of AMF OTU richness and OTU diversity. Instead of interpolating backward to smaller samples, richness estimators extrapolate beyond what has been recorded, to estimate the asymptote of a species accumulation curve (Gotelli and Chao, 2013). Therefore, we used the *iNEXT* function in the iNEXT R package (Hsieh et al., 2016) with intra- and extrapolation to the double of each reference sample size with 40 equally spaced knots. For each root sample, we calculated Hill numbers: Hill N_0 (species richness), Hill N_1 (exponential of Shannon entropy), Hill N_2 (Inverse Simpson concentration). To check whether fields and rice varieties were sequenced deep enough, rarefaction curves of the dataset were generated by using the *rarefaction.single* command in MOTHUR (Fig. S2 and Fig. S3).

Next, we applied generalized linear mixed models (GLMMs) to evaluate the relationships between AMF richness (Hill N_0) and diversity (Hill N_1 and Hill N_2) on the one hand and rice variety (five), rice variety type (HYV vs. traditional) and the soil chemical variables on the other hand (Table S2). Since the rice varieties are nested in rice variety types, rice variety type was added as a nested term in the GLMMs. Furthermore, we also included the variable

'field' as random factor to account for potential spatial autocorrelation. We calculated Variance Inflation Factors (VIF) to detect multicollinearity between the explanatory variables, which were found to be low (<3.1), indicating that all soil chemical variables could be used together in the models. To detect any spatial structuring, we used principal coordinates of neighbour matrices (PCNM) to transform spatial distances to a position vector which was also used as a continuous variable in the models. Therefore, we calculated a set of spatial predictors from the geographical coordinates of the rice fields by using the 'pcnm' function of the R-package *Vegan* (Oksanen, 2017). We deliberately used only the first spatial vector (PCNM1) in the model as it clearly explained the geography of our study area. The response variables Hill N_0 , Hill N_1 and Hill N_2 comprised positive, continuous, right skewed data. Therefore, we modelled these variables using the Gamma probability distribution with inverse link function using 'glmer' function in the *lme4* package. To account for differences in scale and variation of the environmental explanatory variables, they were standardized using the *scale* function in R, which sets the mean of each continuous variable zero and the standard deviation one. To select the most parsimonious model, we used the Akaike Information Criterion (AIC). We calculated estimated marginal means of the final models using the 'emmeans' function in the *emmeans* package and mean comparisons were carried out using the Tukey HSD post-hoc test and t-test, respectively in case 'rice variety' and 'rice variety type' showed significant effects on diversity estimates.

We tested for differences in AMF community composition between the rice varieties and types by performing canonical redundancy analysis (RDA) using the R-package *Vegan*. First, we performed full RDA to relate the effect of soil chemical variables and spatial variables to AMF community composition and structure. Next, we used partial RDA by using the 'condition' statement in the RDA model to control for the variation in soil chemical variables and spatial variables, and then we explicitly tested for effects of the variables 'rice variety' and 'rice variety type'. We performed permutation tests on the individual terms (1000 permutations) using the *anova.cca* function in the *vegan* R package to test the significance of the explanatory variables in the model. Before the full and partial RDA analysis, we standardized explanatory variables using *scale* function in R and the AMF OTU abundance data by Hellinger transformation.

Finally, we performed an indicator species analysis to identify OTUs specific for any of the five rice varieties or two variety types (Dufrene & Legendre 1997). For this analysis, we used the *multipatt* function of the *indicspecies* package in R, with a significance test based on 999 permutations (De Caceres 2013). This analysis provides an

indicator value based on average abundance and relative frequency of the OTUs in each group. We also drew two separate Venn diagrams to show the unique and shared AMF OTUs between the rice varieties and variety types, using the *Venn* package in R.

Results

Sequencing results

Illumina sequencing yielded a total of 4,223,634 reads and 889 OTUs after quality filtering. Among these, 238,759 reads assigned to 75 OTUs, were identified as Glomeromycota after BLAST searching in GenBank and verification against the MaarjAM database at 95%-100% sequence identity. In total, six AMF families were identified (Fig. S1). Glomeraceae was the most abundant family (60%) with 162,957 sequences belonging to 45 OTUs, followed by Claroideoglomeraceae (17.34%, 50,012 sequences of 13 OTUs), Paraglomeraceae (12%, 15,510 sequences of 9 OTUs), Acaulosporaceae (8%, 9,894 sequences of 6 OTUs), Diversisporaceae (1.33%, 277 sequences of 1 OTU) and Gigasporaceae (1.33%, 109 sequences of 1 OTU). All six AMF families were observed in the BR28, Ushapari and Shampakatari varieties with 54 (28,945 sequences), 60 (85,767 sequences) and 53 (85,618 sequences) OTUs, respectively (Fig. 2a and 2b). BR29 and BR58 occurred with four and five AMF families with 42 (28,021 sequences) and 42 (10,361 sequences) OTUs, respectively (Fig. 2a and 2b). Traditional varieties had the highest number of OTUs (70 OTUs, 71,385 sequences) compared to modern HYV (63 OTUs, 673,74 sequences) (Fig. 2c and 2d). The rarefaction curves (OTU richness vs. number of sequences) showed saturation at 73,370, 64,640, 17,800, 23,430 and 8,740 sequence reads for Ushapari, Shampakatari, BR28, BR29 and BR58, respectively (Fig. S2).

AMF richness and diversity

The model selection procedure to relate AMF richness to the environmental variables selected rice variety type ($\chi^2 = 3.00$, $P = 0.051$), rice variety ($\chi^2 = 9.29$, $P = 0.025$), Olsen P ($\chi^2 = 6.58$, $P = 0.010$) and PCNM1 ($\chi^2 = 8.65$, $P = 0.003$) as the explanatory variables (Table 1). Rice variety type ($\chi^2 = 4.53$, $P = 0.033$) and rice variety ($\chi^2 = 9.15$, $P = 0.027$), Olsen P ($\chi^2 = 6.48$, $P = 0.010$) and PCNM1 ($\chi^2 = 11.88$, $P < 0.001$) were significantly related with Shannon diversity. Simpson diversity was significantly explained by rice variety type ($\chi^2 = 4.95$, $P = 0.025$), rice variety ($\chi^2 = 7.81$, $P = 0.049$), Olsen P ($\chi^2 = 3.92$, $P = 0.047$) and PCNM1 ($\chi^2 = 11.28$, $P < 0.001$) (Table 1). Post-hoc pairwise comparisons showed that modern HYV had a lower AMF richness ($P = 0.035$), Shannon diversity ($P = 0.017$) and Simpson diversity

($P = 0.022$) than the traditional varieties (Fig. 3). Pairwise comparisons among the five individual rice varieties revealed that Ushapari had a significantly higher AMF richness than BR29 ($P = 0.042$) and BR58 ($P = 0.012$), while the AMF richness of BR28, BR29, BR58 and Shampakatari did not significantly differ from each other ($P > 0.05$) (Fig. 3). Similar results were found for Shannon and Simpson diversity (Fig. 3).

AMF community composition

RDA showed a significant relationship of soil moisture content ($F = 2.672$, $P = 0.002$), PCNM1 ($F = 3.080$, $P = 0.001$) and rice variety type ($F = 2.420$, $P = 0.001$) (Fig. S4). The partial RDA model showed a significant difference in AMF community composition and structure between modern HYV and traditional rice varieties ($F = 2.584$, $P = 0.001$). The AMF communities of the traditional rice varieties (Ushapari and Shampakatari) differed from those of the modern HYV rice varieties (BR28, BR29 and BR58), despite some overlap (Fig. 4).

Indicator species analysis

The indicator species analysis identified 9 OTUs (5 belonging to *Claroideoglomeraceae*, 3 belonging to *Glomeraceae* and 1 belonging to *Paraglomeraceae*) significantly associated with a traditional rice variety type (Table 2), while no indicator OTUs were found for the modern HYV rice variety type. An indicator species analysis was performed on the five rice varieties, two indicator OTUs were significantly associated with the Ushapari rice variety. These were OTU22 (indicator value = 0.537, $P = 0.001$) (*Glomeraceae*) and OTU114 (indicator value = 0.413, $P = 0.002$) (*Claroideoglomeraceae*). No significant indicator OTU was detected for the other rice varieties.

Unique and shared AMF OTUs

Further analysis to detect unique and shared OTUs between the two rice variety types revealed that 12 OTUs (9 belonging to *Glomeraceae* and 3 belonging to *Claroideoglomeraceae*) were only present in the traditional rice variety type (Table S3). In contrast, there were just 5 OTUs (belonging to *Glomeraceae*) unique to the modern rice variety type (Fig. 5, Table S3). The highest number of unique OTUs was found in the Ushapari variety (7 OTUs), compared to 2 OTUs in BR28, 1 OTU in BR29, 1 OTU in BR58 and 2 OTUs in Shampakatari (Fig. 5). There were 26 OTUs that occurred in all varieties. Ushapari shared a higher number of OTUs with Shampakatari (43 OTUs) and BR28 (45 OTUs) than with BR29 (38 OTUs) and BR58 (37 OTUs).

Discussion

Although AMF are generally considered to have a low host specificity, past studies have demonstrated that AMF diversity and community structure vary across different crop species and even crop genotypes (Turrini et al., 2016). Based on our high throughput amplicon sequencing data, we evaluated the variation among indigenous AMF communities across different rice varieties, which were sampled across 40 fields in the northern part of Bangladesh. This is the first study on the topic in rice. We report highly variable AMF communities across different rice varieties and higher average AMF richness and diversity in the two traditional rice varieties than three modern high yielding varieties.

Illumina sequencing of the SSU rRNA genes fragment revealed overall 75 AMF OTUs from six AMF families, among which Glomeraceae were the most abundant family, irrespective of the rice variety type. This result is in line with a previous study in Bangladeshi paddy rice fields (Parvin et al., 2019). Additionally, Watanarojanaporn et al. (2013), Wang et al. (2015) and Sarkodee-Addo et al. (2020) have documented Glomeraceae as the most prevalent AMF family in roots of rice from paddy fields in Thailand, China and Ghana, respectively. This dominance of Glomeraceae is likely due to their widespread distribution and well known adaptation to grow in both nutrient deficient and enriched soils (Daniell et al., 2001).

We found a significant negative effect of bioavailable phosphate on both AMF richness and diversity. In general, there is a broad agreement that soil phosphorus is one of the most important drivers of AMF occurrence and abundance in agricultural soils (Van Geel et al., 2017; Liu et al., 2020). High soil phosphorus hinders the proliferation of hyphae and diminishes their functionality by suppressing the expression of AMF-related genes, which subsequently change the physiological processes and reduce the susceptibility of plants to AMF (Gu et al., 2011). The spatial predictor (PCNM1) also significantly contributed to AMF richness and diversity. PCNM1 separated the rice fields according to their longitude and may correlate with unmeasured environmental variables such as soil texture, temperature, aeration and inorganic nutrients such as nitrogen. Yet, along with soil phosphorus and the spatial predictor, our model also showed a significant effect of both rice variety and type. Overall, we found high variation in AMF richness and diversity among the studied rice varieties, which is consistent with a very recent study investigating AMF community composition of 13 commonly grown rice varieties in the southwest of Nigeria, based on spore morphology (Olubodea et al., 2020). The variation of AMF communities among different plant species or even different varieties of the same crop is well documented (Dickie, 2007; Burns et al., 2015). Our study further confirmed this phenomenon for different

Bangladeshi rice varieties. Niche-based processes, driven by specific functional traits, may play a key role for the differential interactions between host plants and AMF communities (Chagnon et al., 2012). For example, the development of distinct AMF communities in the rhizosphere of different plant species may be driven by preferential allocation of plant carbon to the most beneficial fungal partner (Bever et al., 2009). Also, the existence of a reciprocal reward mechanism, which stabilizes the cooperation between plants and fungi, allows plants to detect, discriminate, and reward the best fungal partners with more carbohydrates (e.g. Kiers et al., 2011). Moreover, fungal partners enforce cooperation by increasing nutrient transfer only to those roots providing more carbohydrates.

We showed that average AMF diversity was higher in the traditional rice type than in the modern HYV rice type. An investigation of AMF responsiveness over 27 crop species also showed higher AMF colonization in wild progenitor crops, irrespective of phosphorus availability, while domesticated crops only benefited under phosphorus poor conditions, suggesting domestication compromises AMF (Martín-Robles et al., 2018). In agreement with our diversity analysis, the full and partial RDA analysis revealed a significant shift of AMF community composition and structure from traditional rice varieties to the modern HYVs, which is in agreement with a recent study by Garcia de Leon et al. (2020), who also reported a divergence of AMF communities of an old wheat cultivar from those of modern wheat cultivar. Furthermore, the detection of nine indicator AMF OTUs (belonging to Glomeraceae, Claroideoglomeraceae and Paraglomeraceae) in the traditional rice type only suggests a preference of these AMF taxa by traditional rice varieties. Modern breeding coupled with high fertilization provides a plausible explanation for the observed reduced AMF diversity and different community composition and structure in modern HYV crops as compared to traditional varieties (Hetrick et al., 1993; Martín-robles et al., 2020). First, crop varieties differ in architectural features, such as root diameter, length and density, and those differences can not only influence the uptake of soil nutrients, but also community assembly and the function of the AMF root symbiosis (Baon et al., 1994; Maherali, 2014). Although root traits are genetically highly variable, there is no doubt that changes in root architecture, morphology and activity can improve nutrients absorption efficiency (Gahoonia et al., 1999; Hao et al., 2008). Several studies, considering both wild species and crops, suggested that low branched thick fine roots are more suited for colonization and development of AMF (Eissenstat et al., 2015). Increased AMF abundance has been reported with increasing root diameter and decreased specific root length (Sweeney et al., 2020). Furthermore, a meta-analysis has demonstrated that highly branched fibrous root systems hinder effective symbiotic functioning which could be due to the larger absorptive surface area of heavily branched roots, facilitating the uptake of immobile nutrients and

overruling the plant's dependence on AMF (Yang et al., 2015). The modern HYV of rice used for the present study, indeed, have highly branched root systems (Uddin et al., 2009). Second, the penetration of AMF into plant roots also relies on plant symbiosis signaling pathways activated by specific genes (Gutjahr et al., 2008). For rice, Lysin motif receptor-like kinase OsCERK1 is essential for the activation of this signaling pathway and AMF root colonization (Huang et al., 2019). It seems plausible that allelic variants of the OsCERK1 gene in different rice varieties could affect AMF development and symbiotic efficiency (Lefebvre, 2020). Traditional rice varieties perhaps have a higher richness of OsCERK1 than HYVs, resulting in higher AMF diversity and colonization. Furthermore, plant pathogens represent a major threat to crop production. Crop breeding has efficiently helped to mitigate these threats, making modern crop varieties more resistant against pathogens (Khan et al., 2020). However, in most cases this pathogen resistance breeding takes place at the cost of accommodating beneficial microorganisms (Zuccaro and Langen, 2020). For example, the Mildew Resistance Locus O (MLO) regulates the penetration of the host cell wall by powdery mildew fungi, yet it also affects the penetration of AMF hyphae (Jacott et al., 2020). MLO mutant barley and wheat are therefore less colonized by AMF, which is accompanied by a pronounced decrease in the expression of many of the key genes required for intracellular accommodation of AMF (Jacott et al., 2020). Therefore, it is not unlikely that also modern rice varieties have a lower AMF diversity. In addition, the establishment of the AMF symbiosis is also initiated and regulated by a variety of root phytochemicals such as Strigolactones (Biate et al., 2015). Profile of these phytochemicals varies with the crop genotype (Ellouze et al. 2012). Selection of HYVs through breeding programs, which traditionally takes place on fertile soil, may lead to the loss of genes involved in root phytochemicals secretion, hence limiting the formation of an efficient AMF symbiosis (Bazghaleh et al., 2018). Root phytochemical profiling, OsCERK1 and MLO gene expression pattern of the rice varieties studied here may confirm these assumptions. Third, from an evolutionary perspective, artificial selection during breeding could have promoted crop varieties that are less responsive to AMF (Martín-Robles et al., 2018). For instance, selection of HYVs could have limited the translocation of carbohydrates to roots, resulting in a decrease in resources available for AMF. In addition, HYV crops grown under high-fertilization regimes are generally more phosphorus responsive, which might also discourage plants from investing their assimilated carbon in nutrient acquisition via AMF symbiosis (Mäder et al., 2000; Nijjer et al., 2010). Werner and Kiers (2015) also supported this hypothesis by demonstrating that the cultivation history of host plants could have led to selection impairing the ability to choose high-quality/cooperative AMF strains.

However, some other studies, did not find differences of AMF colonization among modern and traditional varieties (An et al., 2010; Lehmann et al., 2012). Considering the fact that AMF dependency of a given plant is not only determined by host plant species but also by fungal partner and the environment, it is still difficult to pinpoint similarities, differences and variations in the behavior of different plant species and cultivars with regard to interactions with AMF. Whereas the observed changes in AMF diversity and community structure in relation to the rice variety type tell us about how AMF communities react to modern breeding practices, we can only hypothesize regarding the precise mechanisms. In order to increase our mechanistic understanding, analysing the meta-transcriptome can provide insight regarding what genes are actively transcribed and whether there are differences among modern and traditional rice varieties (Güimil et al., 2005; Campos et al., 2019; Tian et al., 2019).

Distinguishing different taxa is a crucial step towards the characterization of mycorrhizal communities in the field. However, due to unexplored diversity and lack of reliable annotated sequences, it is not so easy to set clear species delimitation in the kingdom fungi. This is particularly true for AMF with their enormous intraspecific ribosomal DNA (rDNA) sequence variability. Although high-throughput amplicon sequencing technology has revolutionized mycorrhizal community ecology by providing a robust tool to assess biodiversity, there are still some limitations associated with estimating true mycorrhizal species richness using a metabarcoding approach. Several technical biases might arise from PCR and/or sequencing platform itself. For instance, the primer pair (AMV4.5NF-AMDGR) used to study AMF communities delivers high quality AMF sequences but favors amplification of Glomeraceae sequences at the expense of Ambioporceae, Claroideoglomeraceae and Paraglomeraceae sequences, and can sometimes also produce a high number of non-Glomeromycota sequences (Van Geel et al., 2014). For this reason, in the present study, it is possible that some root samples might have not yielded sufficient AMF sequence reads for their inclusion in the analyses. Additionally, the Illumina Miseq platform is also known to exhibit a methodological bias, artificially inflating or underestimating the number of OTUs found (Brown et al., 2015). To account for these biases, the downstream processing of the obtained microbial sequences and estimating microbial species richness should be done with great care. In our specific case, the rarefaction curves of two of the studied rice varieties (BR29 and BR58) showed no complete saturation, suggesting that these varieties may not have been sampled adequately. However, we believe that through using the iNEXT extrapolation, we dealt with unequal sequencing depth, providing reliable and comparable measures of AMF OTU richness and diversity. In addition, we also did not find significant differences in

the number of sequence reads among the five varieties (Fig. S5). Therefore, we are confident that observed differences in AMF diversity and community composition among the studied rice varieties were not an artifact.

Conclusions

Our results revealed that AMF diversity and community composition differed substantially among the five studied rice varieties, suggesting existence of partner preference on either side of the symbiosis. Our data also showed significant variation of AMF diversity and, community composition and structure between modern HYV and traditional rice variety types, indicating the possibility of genetic selection having inadvertently altered the ability of modern varieties to benefit from services naturally provided by the soil microbes, such as AMF. Although AMF colonization is not only determined by host plant species but also by the environment and AMF abundance in soil, the intraspecific variation observed among the different rice varieties points at potential benefits of selecting rice genotypes that can form an efficient symbiosis with naturally occurring AMF, especially in terms of required external agricultural inputs.

Declarations

Funding

This research was financially supported by IRO, KU Leuven's Interfaculty Council for Development Cooperation.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

SP, MVG and OH designed the study. SP performed the field sampling, lab analyses, analyzed the data and wrote the first draft. MMA and TY assisted with field sampling and sample transportation. MVG and OH assisted with the data analysis. MVG, OH, BL and MMA edited the manuscript. All authors gave final approval for publication.

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Figure captions

Fig. 1 Location of the 40 rice fields in the Dinajpur District in the Northern part of Bangladesh. Different colored pins indicate different rice varieties

Fig. 2 Relative proportion of operational taxonomic units (OTU) of arbuscular mycorrhizal fungal (AMF) families (a) and their relative family read abundances (b) in roots of five Bangladeshi rice varieties (BR28, BR29, BR58, Shampakadari and Ushapari) of a total of 40 fields ($N = 144$) at harvesting stage. Relative proportion of AMF OTU families (c) and their relative family read abundances (d) in the modern high-yielding and traditional varieties. Absolute number of OTUs and read abundances are shown on the top of the bar

Fig. 3 Comparison of arbuscular mycorrhizal fungal (AMF) richness (Hill N_0), Shannon diversity (Hill N_1) and Simpson diversity (Hill N_2) among five Bangladeshi rice varieties (BR28, BR29, BR58, Shampakadari and Ushapari) and two rice variety types (Modern HYV vs Traditional), sampled from 40 fields ($N = 144$) at harvesting stage. Bars represent estimated marginal means with standard errors, obtained from Generalized linear mixed models (GLMMs). Different letters indicate significant differences among rice varieties based on a Tukey HSD post-hoc test. ‘*’ indicates a significant difference among modern and traditional rice variety types at $P < 0.05$ based on a t-test

Fig. 4 Partial redundancy bi-plot of the arbuscular mycorrhizal fungal (AMF) communities of five Bangladeshi rice varieties (BR28, BR29, BR58, Shampakadari and Ushapari) sampled from 40 rice fields ($N = 144$) at harvesting stage. The red squares indicate the centroids of the AMF communities of the rice varieties and the confidence (95% level) ellipses highlight the shift in the AMF communities between modern high yielding (solid black line) and traditional rice varieties (dashed black line). RDA1 and RDA2 axis account for 45.9% and 31.6% of the total explained variance, respectively

685 **Fig. 5** Venn diagram showing the number of unique and shared arbuscular mycorrhizal fungal (AMF) operational
686 taxonomic units (OTUs) among Bangladeshi rice (*Oryza sativa* L.) breeding types (a) and individual rice varieties (b)