Distribution of arbuscular mycorrhizal fungi in sugarcane rhizosphere from various agricultural management practices in Northeast, Thailand

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Abstract
Sugarcane (Saccharum spp.) is one of the economically most important crops in Thailand. Sugarcane forms symbiotic associations with arbuscular mycorrhizal fungi (AMF). Species diversity of and root colonization by AMF may vary by agricultural management and soil properties. The objective of this study was to investigate the community composition of AMF in sugarcane rhizosphere soil with various cultivation practices. Twelve sugarcane rhizosphere soils were collected from sugarcane fields in three provinces (Nakhon Ratchasima, Khon Kaen, and Buri Ram) with various forms of agricultural management, including organic farming (OM), semi-organic farming (SM), and conventional farming with mineral fertilizers (CM). The results showed that root colonization ranged between 10 and 22%, while spore density ranged from 11 to 168 spores 100 g soil⁻¹. Based on morphological identification of AMF, a total of 43 taxa, representing 11 genera, were observed, viz. the genera Acaulospora, Claroideoglomus, Dentiscutata, Diversispora, Entrophospora, Funneliformis, Gigaspora, Glomus, Racocetra, Rhizophagus, and Septoglomus. One unidentified species was found. The dominant genera were Acaulospora and Glomus, which were found in all sites. Diversispora pustulata was the most widely distributed species, isolated in 75% of the sites. Species diversity of AMF, expressed by Shannon–Wiener index of diversity (H’), ranged from 1.03 to 2.14 with the highest diversity in OM systems and lowest diversity in CM systems. Our results may be used for considerations of agricultural management practices to benefit from communities of native AMF, which could be important for sustainable production of sugarcane.

Keywords – Agricultural management – Arbuscular mycorrhizal fungi – Diversity – Sugarcane

Introduction
Sugarcane (Saccharum spp.) is one of the economically most important crops in countries located in tropical and subtropical regions. Sugarcane is the major raw material for sugar production. Global sugar production for 2019/20 was estimated at 174 million tons (USDA 2019). Thailand is one of the global leaders in the sugarcane industry as the fourth largest producer and second largest sugar exporter (Chunhawong et al. 2018). Most of the sugarcane cultivation in Thailand occurs in northeast Thailand. Sugarcane is cultivated in different agricultural management
systems. Part of sugarcane is grown organically, which is known to have a range of benefits for plants and the environment (Oliveira et al. 2017). On the other hand, some farmers rely on conventional farming with substantial inputs of mineral fertilizers and synthetic biocides which may affect soil microbiota (Hartmann et al. 2015).

Arbuscular mycorrhizal (AM) fungi (phylum Glomeromycota) are a group of important soil microorganisms. AM fungi form obligate symbiotic associations with more than 80% of terrestrial plants (Smith & Read 2008). Currently, approximately 334 AM fungal species have been reported globally (AMF – Phylogeny 2019). They play important roles in agroecosystems because they enhance the uptake of water and nutrients, particularly immobile nutrients like phosphorus (P) by extension of their extramatrical mycelium in the soil beyond the depletion zone (Aroca et al. 2007, Smith & Read 2008, Sharif & Claassen 2011, Bhardwaj et al 2014). Several AM fungal species are known to be associated with sugarcane, and the benefits of these species for the growth of sugarcane have been reported (Rokni & Goltapeh 2011, Kumalawati et al. 2014, Pontes et al. 2017). Other previously also reported that the combined application of AM fungal inoculum and rock phosphate increased cane yield by 51% compared to control treatments (Juntahum & Boonlue 2018). However, the benefits of AM fungi on plant growth also depend on their community composition (Neuenkamp et al. 2019).

Communities of indigenous AM fungi in sugarcane have been studied in several countries. Those studies focused on sugarcane varieties, different soil types and, seasonal variation in community composition (Rokni & Goltapeh 2011, Datta & Kulkarni 2012, Suresh & Nelson 2015). Up to now, no studies have been conducted in Thailand. Since agricultural practices may affect microbial abundance in soils. The cultivation of sugarcane then needs proper management systems to keep soil fertility throughout the seasons, especially in northeast Thailand, where the soils in agricultural areas are mainly sandy soils and poor in organic matters and nutrients. Regarding to this, the 3 areas of selection, Nakhon Ratchasima, Khon Kaen, and, Buriram, are the ones that have been constantly conducted a single management either organic farming, semi-organic farming, or conventional farming with mineral fertilizer for years due to their contract farming with sugar mills. Therefore, these areas are good representatives for the study of the distribution of AMF in the sugarcane rhizosphere collected from different agricultural managements. In addition, the previous studies did not yet include various forms of more intensive (conventional) or more extensive (organic) agricultural systems. The agricultural management system likely influences AM fungal community composition. Therefore, the aim of this study was to investigate community composition, diversity, and spore abundance of AM fungi in sugarcane rhizosphere soil from fields with various cultivation practices in the northeast of Thailand.

Materials & Methods

Collection of root and soil samples

Soil samples were collected from differently managed sugarcane fields including fields under organic farming (OM), semi-organic farming (SM), and conventional farming (CM), which their agricultural management was shown in Table 1. Fields located in three provinces in Thailand, viz. Nakhon Ratchasima (NMA), Khon Kaen (KKN), and Buri Ram (BRM) province (Table 1, Fig. 1). All sites that were investigated were under sugarcane KK3 variety monoculture for at least 3 years. Series of sampling of sampling site was reported that Ban Phai - Nam Phong series (sandy soil and pH 5.5-6.5) for NMA, Nam Phong series (sandy soil and pH 5.5-6.0) for KKN, and Buri Ram (BRM) province (Table 1, Fig. 1). Soil samples were collected in August 2015, the rainy season. The average annual precipitation and temperature of NMA, KKN, and BRM were 1,163.7, 1,229.1, and 1,003.7 mm and 27.85, 27.90, and 27.68 °C, respectively. Rhizosphere soils were in 10 random areas with a dimension of 3x3 m were sampled from each sugarcane field. A 5.8 inch open-face auger was used to collect soil samples into the depth of 0-20 cm away from the soil surface. Soil samples of the same field were mixed well before sub-sampling for 500-700 g in order to obtain a composite soil sample for each field. The samples were then kept in plastic bags until
After that rhizosphere soils were air-dried, and then root and soil were separated. Soil samples were used for isolation and subsequent quantification of AM fungal spores, while root samples were used for the determination of root colonization. The soil chemical properties were sent for chemical analysis (organic matter content, availability of nitrate, phosphorus, and potassium) to the laboratory of the Department of Plant Science and Agricultural Resource, Faculty of Agriculture, Khon Kaen University (Table 2). The total organic matter was measured by the wet oxidation method according to Walkley & Black (1934). Available nitrate (NO$_3^-$) was extracted by 1 M KCl, then it was quantified by the Flow Injection Analyzer. Available P in soil was determined by Bray–II according to Bray & Kurtz (1945). The exchangeable K was extracted by ammonium acetate, then it was quantified by a flame photometer.

**Fig. 1** – Locations of sampled sites (arrowhead) in Northeast, Thailand.

**Assessment of AM fungal root colonization**
Roots were stained according to the method of (Koske & Gemma 1989). For this, root samples were washed well by tap water and cleared in 2.5% KOH for 1 h at 90 °C, acidified with 1% HCl, and stained for 8 h or overnight in acetic glycerin solution with 0.05% Trypan blue. The density of AM fungal colonization was scored under a compound microscope in five classes according to the method described by Trouvelot et al. (1985) and subsequently transformed into a percentage of AM fungal colonization.

**Quantification and isolation of AM fungal spore**
The spore number in 100 grams of soil was determined after thoroughly extracting and separating spores from the soil by the wet sieving and decanting technique (Gerdemann & Nicolson 1963). For this, a suspension of soil in water was decanted through a series of sieves with the following aperture, 250 µm, 125 µm, 90 µm, and 63 µm, stacked from top to bottom, respectively. The AM fungal spores collected in each of the sieves were collected in Petri dishes and counted under a stereomicroscope.

**Identification of AM fungal species**
AM fungal spores were mounted on slides containing a drop of polyvinyl alcohol-lactic acid-glycerol (PVLG) with and without Melzer's reagent for the identification of AM fungal species. AM fungal species were identified based on spore morphological characteristics and the species descriptions following the Manual for the identification of VA mycorrhizal fungi (Schenck & Pérez 1990), the Species descriptions from reference cultures (INVAM 2019), and the Glomeromycota
TAXONOMY (AMF – phylogeny 2019). Spores that could not be identified to species level were identified to genus level and indicated under the generic name with a number.

Quantification and isolation of AMF spore

The spore number in 100 grams of soil was determined after thoroughly extracting and separating spores from the soil by the wet sieving and decanting technique (Gerdemann & Nicolson 1963). For this, a suspension of soil in water was decanted through a series of sieves with the following aperture, 250 µm, 125 µm, 90 µm, and 63 µm, stacked from top to bottom, respectively. The AMF spores collected in each of the sieves were collected in petri dishes and counted under a stereomicroscope.

Table 1 Geographical location and field conditions of sampling sites with different types of agricultural management; organic farming (OM), semi-organic farming (SM), and conventional farming (CM).

| Fields | Province of soil sampling sites | Soil sampling area (m²) | Geography | Fertilizing management |
|--------|--------------------------------|------------------------|-----------|-----------------------|
|        |                                |                        | Latitude  | Longitude             | Mineral Fertilizing (kg ha⁻¹) | N  | P₂O₅ | K₂O | Years of practice |
| SM1    | NMA                            | 15,689.00              | 15°43'19.6"N 102°17'02.7"E | Compost 3125 (kg ha⁻¹) | 190.63 | 46.88 | 46.88 | 8 |
| SM2    | NMA                            | 15,293.09              | 15°40'59.0"N 102°21'28.3"E | Compost 3125 (kg ha⁻¹) | 190.63 | 46.88 | 46.88 | 3 |
| SM3    | NMA                            | 10,807.60              | 15°43'28.7"N 102°16'22.0"E | Rice Straw + Compost 3125 (kg ha⁻¹) | 143.75 | 0 | 0 | 5 |
| SM4    | NMA                            | 15,932.83              | 15°43'36.2"N 102°17'02.5"E | Compost 3125 (kg ha⁻¹) | 143.75 | 0 | 0 | 5 |
| SM5    | NMA                            | 21,196.45              | 15°36'43.5"N 102°12'41.5"E | Compost 6250 (kg ha⁻¹) | 143.75 | 0 | 0 | 3 |
| SM6    | NMA                            | 18,482.48              | 15°40'45.6"N 102°12'14.0"E | Compost 3125 (kg ha⁻¹) | 143.75 | 0 | 0 | 6 |
| SM7    | NMA                            | 19,303.35              | 15°43'24.5"N 102°16'43.3"E | Compost 6250 (kg ha⁻¹) | 143.75 | 0 | 0 | 5 |
| SM8    | BRM                            | 22,545.75              | 15°16'15.1"N 103°05'13.3"E | Green manures, sunn hemp + Compost 6250 (kg ha⁻¹) | 46.88 | 46.88 | 46.88 | 3 |
| OM1    | NMA                            | 22,283.80              | 15°38'23.2"N 102°12'23.9"E | Compost 12500 (kg ha⁻¹) + Rick husk 125 (kg ha⁻¹) + Manure 1250 (kg ha⁻¹) + Biofertilizer | 0 | 0 | 0 | 5 |
| OM2    | NMA                            | 12,324.99              | 15°43'15.6"N 102°17'02.7"E | Rice Straw + Compost 3125 (kg ha⁻¹) + Manure 1250 (kg ha⁻¹) + Swine manure fermented | 0 | 0 | 0 | 5 |
| CM1    | NMA                            | 17,468.36              | 15°43'16.3"N 102°17'08.4"E | - | 46.88 | 46.88 | 46.88 | 10 |
| CM2    | KKN                            | 24,636.23              | 16°42'32.3"N 102°55'18.1"E | - | 143.75 | - | - | 5 |

Note: The province of each sampling site was Nakhon Ratchasima (NMA), Khon Kaen (KKN), and Buri Ram (BRM).
Table 2 Soil chemical properties of sampling sites with different forms of agricultural management; organic farming (OM), semi-organic farming (SM), and conventional farming (CM).

| Fields | Organic matter (%) | NO\text{\textsubscript{3}} (ppm) | Available P (ppm) | Exchangeable K (ppm) |
|--------|---------------------|----------------------|-------------------|---------------------|
| SM1    | 0.47                | 6.66                 | 50.75             | 38.02               |
| SM2    | 0.83                | 15.90                | 34.75             | 63.69               |
| SM3    | 0.95                | 9.20                 | 31.25             | 90.30               |
| SM4    | 0.89                | 11.27                | 49.38             | 62.74               |
| SM5    | 1.58                | 10.93                | 360.00            | 91.25               |
| SM6    | 0.73                | 11.92                | 295.13            | 85.55               |
| SM7    | 0.67                | 3.86                 | 241.00            | 47.53               |
| SM8    | 1.04                | 2.81                 | 662.00            | 115.02              |
| OM1    | 1.44                | 10.24                | 213.50            | 61.79               |
| OM2    | 1.03                | 11.62                | 149.50            | 118.82              |
| CM1    | 0.62                | 9.56                 | 8.95              | 36.12               |
| CM2    | 0.92                | 6.70                 | 9.10              | 29.47               |

AM fungal species distribution

The AM fungal communities were expressed following (Shi et al. 2006) including; Species richness (SR): the number of morphologically separate AM fungal species in each soil sample; Spore density (SD): the number of spores in 100 gram soil; Relative abundance (RA): the relative number of AM fungal spores per species or genus; Isolation frequency (IF): the fraction of sites where the AM fungal species or genus was observed; Simpson’s Index of Diversity (D’): calculated by the formula D’ = \( \sum_{i=1}^{s} P_i^2 \); and Shannon –Wiener’s Index of diversity (H’): calculated by formula H’ = - \( \sum_{i=1}^{s} P_i \ln P_i \), where \( i \) is the individual species, and \( P \) is the proportion of spores belonging to the \( i^{th} \) species.

Statistical analysis

Linear correlation was applied to assess relationships between soil properties root colonization and properties of the AM fungal community. The analysis was performed based on Pearson’s correlation coefficient using Statistix program version 8.0.

Results

AM fungal colonization

AM fungal colonization differed amongst different management types. AM fungal colonization in sugarcane roots ranged from 10 to 22% (Table 3). Root colonization was significantly higher in both organically managed fields than in the other fields (P < 0.001). Root colonization was marginally significantly correlated with the organic matter content of the fields, but not with other soil properties (Table 5). Moreover, root colonization was correlated with Shannon – Wiener index of diversity and species richness (Table 5).

Spore abundance and AM fungal species composition and diversity

Spore abundance in sugarcane rhizosphere soil ranged from 11-168 spores 100 g soil\textsuperscript{-1}. with the highest in SM3 (Table 3). Both conventionally managed fields CM1 and CM2 contained the lowest number of spores, but due to large variation in fields, the differences were not significant. Spore numbers were also not significantly correlated with soil properties.

Based on spore morphology, a total of 11 genera representing 43 AM fungal species were identified (Table 4, Fig. 2). The species richness ranged from 4 to 13 species per site. It was slightly but significantly higher (P = 0.04) in both organically managed fields OM1 and OM2 (with 11 and 13 identified species) than in the other systems (species numbers ranging from 4 to 11). Species richness was significantly positively correlated (P = 0.01) with soil organic matter content (Table 5). All 34 species were recorded from eight semi-organically managed sites, 21 species from
two organically managed sites, and 8 species from two conventionally managed sites. Amount of all species, 3 species were found in all management viz *Acaulospora delicata*, *Ac. denticulate*, *Diversispora pustulata*. 19 species were only found in semi-organically managed type viz *Ac. foveata*, *Ac. tuberculate*, Acaulospora sp.1, Acaulospora sp.3, *Claroideoglomus etunicatum*, *Cl. luteum*, Denticutata herterogama, Entrophospora inferquens, *En. nevadensis*, Entrophospora sp.1, *Funneliformis badia*, *Glomus sp.1*, *Glomus sp.4*, *Glomus sp.8*, *Glomus sp.10*, *Glomus sp.14*, *Glomus sp.15*, *Rhizophagus sinuosus* (Table 3). 7 species were only found in organically managed type viz *Acaulospora sp.4*, *Di. tortuosa*, *Fu. mossea*, *Glomus sp.6*, *Glomus sp.12*, *Glomus sp.13*, *Septoglomus constrictum* (Table 3). 2 species were only found conventionally managed type viz *Gigaspora gigantea*, *Glomus sp.7* (Table 3). The most frequent species were *Di. pustulata* (in 75%) of the sites, followed by *Ac. delicata* (67% of sites) and *Ra. fulgida* (50% of sites). The genera *Glomus* and *Acaulospora* occurred in all sites (Table 4).

Species diversity of AM fungi expressed by Simpson’s Index of Diversity (D) and ranged from 0.13 to 0.45 (Table 3). There were no significant differences between management systems in terms of D. Species diversity of AM fungi expressed by Shannon–Wiener index of diversity (H) ranged from 1.03 to 2.14 (Table 3). There were no significant differences in Shannon-Wiener diversity between different management types. Shannon-Wiener diversity was marginally significantly positively correlated (P = 0.06) with soil organic matter content (Table 5), and correlations with other soil properties were not significant.

**Table 3** Percentage of AM fungal colonization, spore density, species richness, and diversity (Simpson and Shannon-Wiener) of AM fungi isolated in sugarcane rhizosphere soil in fields with different agricultural management, including organic farming (OM), semi-organic farming (SM), and conventional farming (CM).

| Fields | Root colonization (%) | Spore density (spores 100 g⁻¹) | Species richness | Simpson’s Index of Diversity | Shannon-Wiener’s Index of Diversity |
|--------|------------------------|---------------------------------|------------------|----------------------------|-----------------------------------|
| SM1    | 10                     | 18                              | 7                | 0.22                       | 1.72                              |
| SM2    | 11                     | 70                              | 8                | 0.23                       | 1.72                              |
| SM3    | 12                     | 168                             | 11               | 0.13                       | 2.14                              |
| SM4    | 12                     | 43                              | 8                | 0.37                       | 1.45                              |
| SM5    | 12                     | 67                              | 11               | 0.19                       | 1.93                              |
| SM6    | 11                     | 16                              | 5                | 0.32                       | 1.35                              |
| SM7    | 11                     | 25                              | 7                | 0.39                       | 1.35                              |
| SM8    | 13                     | 16                              | 5                | 0.26                       | 1.46                              |
| OM1    | 21                     | 70                              | 13               | 0.22                       | 1.91                              |
| OM2    | 22                     | 48                              | 11               | 0.18                       | 1.97                              |
| CM1    | 10                     | 11                              | 4                | 0.45                       | 1.03                              |
| CM2    | 13                     | 20                              | 7                | 0.24                       | 1.64                              |

**Table 4** Spore density (per species) and isolation frequency (IF) of individual AM fungal species in sugarcane rhizosphere soil under different forms of agricultural management, organic farming (OM), semi-organic farming (SM), and conventional farming (CM).

| AM fungal species | Spore density (spores 100 g⁻¹) | IF |
|-------------------|---------------------------------|----|
|                   | SM1    | SM2    | SM3    | SM4    | SM5    | SM6    | SM7    | SM8    | OM1    | OM2    | CM1    | CM2    | |
| *Acaulospora*     | 10     | 38     | 65     | 28     | 14     | 9      | 1      | 2      | 5      | 8      | 2      | 15     | 100    |
| *Ac. delicata*    | 7      | 7      | 29     | 25     | 8      | 1      |        |        |        | 2      | 2      | 2      | 67     |
| *Ac. denticulata* |        |        |        |        |        |        |        |        |        |        |        |        | 25     |
| *Ac. foveata*     |        |        |        |        |        |        |        |        |        |        |        |        | 8      |
| *Ac. mellea*      | 2      |        |        |        |        |        |        |        |        |        |        |        | 6      |
| *Ac. scrobiculata*| 32     | 10     |        |        |        |        |        |        |        |        |        |        | 25     |
| *Ac. tuberculata* | 1      | 1      |        |        |        |        |        |        |        |        |        |        | 17     |
| AM fungal species       | Spore density (spores 100 g\(^{-1}\)) | IF |
|-------------------------|---------------------------------------|----|
|                         | SM1 | SM2 | SM3 | SM4 | SM5 | SM6 | SM7 | SM8 | OM1 | OM2 | CM1 | CM2 |
| Acaulospora sp.1        | 1   |     |     |     |     |     |     |     |     |     |     |     |
| Acaulospora sp.2        | 2   | 6   | 2   | 3   | 1   |     |     |     |     |     |     |     |
| Acaulospora sp.3        | 23  | 3   | 1   |     |     |     |     |     |     |     |     |     |
| Acaulospora sp.4        |     | 1   | 2   |     |     |     |     |     |     |     |     |     |
| Claroideoglomus         |     | 6   |     |     |     |     |     |     |     |     |     |     |
| Cl. etunicatum          |     | 5   |     |     |     |     |     |     |     |     |     |     |
| Cl. luteum              |     | 1   |     |     |     |     |     |     |     |     |     |     |
| Denticulata             | 2   |     |     |     |     |     |     |     |     |     |     |     |
| De. heterogama          | 2   |     |     |     |     |     |     |     |     |     |     |     |
| Diversispora            | 21  | 6   | 3   | 17  | 2   | 6   | 14  | 1   | 1   | 75  |     |     |
| Di. pustulata           | 21  | 6   | 3   | 17  | 2   | 6   | 12  | 1   | 1   | 75  |     |     |
| Di. tortuosa            |     | 2   |     |     |     |     |     |     |     |     |     |     |
| Entrophospora           | 1   | 15  |     |     |     |     |     |     |     |     |     |     |
| En. infrequens          |     | 14  |     |     |     |     |     |     |     |     |     |     |
| En. nevadensis          |     |     | 15  |     |     |     |     |     |     |     |     |     |
| Entrophospora sp.1      |     |     |     |     |     |     |     |     |     |     |     |     |
| Funneliformis           |     | 15  | 2   | 3   |     |     |     |     |     |     |     |     |
| Fu. badia               |     | 15  |     |     |     |     |     |     |     |     |     |     |
| Fu. mosseae             |     |     | 2   | 3   |     |     |     |     |     |     |     |     |
| Gigaspora               |     |     |     |     |     |     |     |     |     |     | 1   | 8   |
| Gi. gigantea            |     |     |     |     |     |     |     |     |     |     |     | 8   |
| Glomus                  | 2   | 9   | 43  | 12  | 8   | 2   | 4   | 4   | 32  | 22  | 7   | 1   |
| Gl. microcarpum         |     | 9   | 4   |     | 1   |     |     |     |     |     |     | 25  |
| Glomus sp.1             |     |     |     |     |     |     |     |     |     |     |     |     |
| Glomus sp.2             |     |     |     |     |     |     |     |     |     |     |     |     |
| Glomus sp.3             |     | 3   | 1   |     |     |     |     |     |     |     |     |     |
| Glomus sp.4             |     | 2   |     |     |     |     |     |     |     |     |     |     |
| Glomus sp.5             |     | 6   |     |     |     |     |     |     |     |     |     |     |
| Glomus sp.6             |     | 4   | 2   | 13  |     |     |     |     |     |     |     |     |
| Glomus sp.7             |     | 1   |     |     |     |     |     |     |     |     |     | 17  |
| Glomus sp.8             |     | 3   |     |     |     |     |     |     |     |     |     |     |
| Glomus sp.9             |     | 4   | 2   |     |     |     |     |     |     |     |     |     |
| Glomus sp.10            |     | 1   |     |     |     |     |     |     |     |     |     |     |
| Glomus sp.11            |     | 3   | 2   |     |     |     |     |     |     |     |     |     |
| Glomus sp.12            |     |     | 24  |     |     |     |     |     |     |     |     |     |
| Glomus sp.13            |     | 1   |     |     |     |     |     |     |     |     |     |     |
| Glomus sp.14            |     | 4   |     |     |     |     |     |     |     |     |     |     |
| Glomus sp.15            |     |     |     |     |     |     |     |     |     |     |     |     |
| Racocetra               | 2   | 2   | 1   | 21  | 1   | 2   |     |     |     |     |     |     |
| Ra. fulgida             | 2   | 2   | 1   | 21  | 1   | 2   |     |     |     |     |     |     |
| Rhizophagus             | 25  | 20  | 3   | 2   | 3   | 6   |     |     |     |     |     |     |
| Rh. clarus              | 20  | 3   |     | 2   |     |     |     |     |     |     |     |     |
| Rh. intraradices        | 25  | 2   |     |     | 4   |     |     |     |     |     |     |     |
| Rh. sinuosus            | 3   |     |     |     |     |     |     |     |     |     |     |     |
| Septoglomus             | 1   | 4   |     |     | 1   |     |     |     |     |     |     |     |
| Se. constrictum         |     |     |     |     |     |     |     |     |     |     | 17  |
| Se. viscosum            |     | 1   | 4   |     |     |     |     |     |     |     |     |     |
| Unidentified            | 1   |     |     |     |     |     |     |     |     |     |     | 17  |
Figure 2 – AM fungal species in sugarcane rhizosphere soils under different types of agricultural management. AM fungal spores are *Acaulospora delicata* (A), *Ac. denticulata* (B), *Ac. foveata* (C), *Ac. mellea* (D), *Ac. scrobiculata* (E), *Ac. tuberculata* (F), *Acaulospora* sp.1 (G), *Acaulospora* sp.2 (H), *Acaulospora* sp.3 (I), *Acaulospora* sp.4 (J), *Claroideoglomus etunicatum* (K), *Cl. luteum* (L), *Denticutata heterogama* (M), *Diversispora pustulata* (N), *Di. tortuosa* (O) *Entrophospora*
infrequens (P), En. nevadensis (Q), Entrophosphora sp.1 (R), Funneliformis badia (S), Fu. mosseae (T), Gigaspora gigantea (U), Glomus microcarpum (V), Glomus sp.1 (W), Glomus sp.2 (X),
Glomus sp.3 (Y), Glomus sp.4 (Z), Glomus sp.5 (AA), Glomus sp.6 (AB), Glomus sp.7 (AC),
Glomus sp.8 (AD), Glomus sp.9 (AE), Glomus sp.10 (AF), Glomus sp.11 (AG), Glomus sp.12
(AH), Glomus sp.13 (AI), Glomus sp.14 (AJ), Glomus sp.15 (AK), Racocetra fulgidula (AL),
Rhizophagus clarus (AM), Rh. intraradices (AN), Rh. sinuosus (AO), Septoglomus constrictum
(AP), Se. viscosum (AQ) and Unidentified 1 (AR). The scale bar is 50 µm lengths.

Table 5 Pearson’s correlation coefficient (r) between soil properties and AM fungal diversity.

| Parameter                  | Exchanging K | NO₃⁻ | Organic matter | Available P | Root colonization | Spore density | H   | D  |
|----------------------------|--------------|------|----------------|-------------|-------------------|---------------|------|----|
| Organic matter             |              |      |                |             |                   |               |      |    |
| NO₃⁻                      | 0.11         | 0.18 |                |             |                   |               |      |    |
| Available P                | 0.62**       | 0.43 | 0.40           |             |                   |               |      |    |
| Root colonization          | 0.42         | 0.15 | 0.55*          | 0.11        |                   |               |      |    |
| Spore density              | 0.31         | 0.32 | 0.38           | -0.23       | 0.16              |               |      |    |
| H                          | 0.40         | 0.25 | 0.56*          | -0.08       | 0.50*             | 0.74**        |      |    |
| D                          | -0.46        | -0.17| -0.49          | -0.05       | -0.41             | -0.63**       | -0.94**|    |
| Species richness           | 0.30         | 0.33 | 0.71***        | -0.11       | 0.67**            | 0.69**        | 0.87**| -0.69**|

The r values were considered for a relationship statistically significant at P≤0.10 (*) and P≤0.05 (**), respectively.

Discussion

Our study represents the first report in Thailand that described and compared AM fungal community structure in commercial (conventional), semi-organic and organic sugarcane fields. In this work, identification of AM fungi was conducted based on their morphology compared to the INVAM database (Schenck & Pérez 1990, INVAM 2019, AMF – phylogeny 2019). Note that although the identification of AM fungi based solely on their morphology is a classical method, this technique has constantly been applied in many peer-reviewed research to date (i.e., Songachan & Kayang 2011, Khaeckhum et al. 2017, Xavier Martin & Rodrigues 2020). As a result, a total of 11 genera representing 43 AM fungal species were identified (Fig. 2, Table 5). The genera Acaulospora and Glomus were most widely distributed, whereas among the species Di. pustulata was most widely distributed, occurring in 75% of the fields. Both Acaulospora and Glomus are commonly found in agricultural systems (Dandan & Zhiwei 2007, Li et al. 2007, Maia et al. 2010).

Note that the aim of this work was to investigate the distribution of AM fungi in rhizosphere soils of sugarcane variety KK3, in which the sugarcane has been cultivated in the fields as a monoculture for at least 3 consecutive years. However, due to sugarcane price volatility and unpredictable impacts of climate change, small-scale farmers tended to change their agricultural practices including the introduction of crop rotation into their farms. As a result, there have been only limited areas where farmers still cultivate sugarcane as a monoculture and also apply the same practice for longer than 3 years. This is the reason why the investigation in this work was conducted with relatively limited sample size and number. Nevertheless, the findings in this current work would adequately provide insight into the effects of different farming practices on the AM fungi community structure.

Organically managed sugarcane fields tended to have a higher rate of root colonization, species richness, and diversity of AM than conventionally managed fields (Table 3). Higher organic matter content was correlated with higher species richness and diversity of AM fungal (Table 5) and tended to higher root colonization (Table 3). The application of organic manures positively influenced AMF population and diversity (Douds & Reider 2003, Borie et al. 2008). There was a previous report indicated that organic management increased the biomass of bacteria, saprotrophic fungi, and AM fungi (Martínez-García et al. 2018). It was found that the growth of
AM fungal hyphae such as *Gl. intraradices* positively correlated with organic compounds released by other microorganisms through the decomposition of organic matters in soils (Gryndler et al. 2009). Moreover, different AM fungal communities were a result of different organic matter compositions in soils and vice versa. In this regard, AM fungi were found to play an important role in the cycling of nutrients in soils through uptake of organic matters (Govindarajulu et al. 2005, Jin et al. 2005). The research by Jan et al. 2014 and Juntahum et al. 2020 showed that inoculation of AM fungi could increase N, P, and micronutrients contents in soils. This suggested that AM fungal population and diversity could improve soil nutrients for plants. In addition, both primary and secondary ones released via decomposition of organic matters had a significant effect on the diversity of AM fungi in soils (Gryndler et al. 2009, Zhu et al. 2016). Therefore, organic farming practice positively affected AM fungal population, which thus resulted in an improvement of soil nutrients.

Sugarcane requires a long-developmental duration and produces a large amount of biomass. Thus, it removes a significant amount of nutrients from the soil. Increasing the acquisition of nutrients through AM fungal management might therefore reduce fertilizer needs for the next plant generation. The uses of environmentally friendly agricultural practices, including organic farming, are more suitable for AM fungal communities (Lee et al. 2008). However, agricultural management practices should be included in a study that aims at obtaining an overview of AM fungal diversity in certain crops. Other management factors such as tillage, fertilizer management, application of herbicides and pesticides, and monocropping versus intercropping also need to be included in future studies. The large variation in semi-organically managed fields suggested a more environmentally friendly way to manage sugarcane production in large-scale plantations. In other words, the inputs of organic matter in both organic farming and semi-organic farming would result in a high AM fungal population and diversity and thus yield sustainable sugarcane production.

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