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FULL PAPER

Diversity and efficiency of arbuscular mycorrhizal fungi in soils from organic chili (*Capsicum frutescens*) farms

S. Boonlue · W. Surapat · C. Pukahuta · P. Suwanarit · A. Suwanarit · T. Morinaga

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Abstract No previous studies have been conducted on the diversity and population of arbuscular mycorrhizal fungi (AMF) in relation to organically grown chili (*Capsicum frutescens* L.) in Thailand. This study was carried out to investigate the diversity and status of AMF populations at four organically managed farms in Ubon Ratchathani and Sisaket provinces. The effects of each AMF species on the growth and nutrient uptake of chili grown in sterile, organically managed soil were determined. Fourteen AM fungal taxa belonging to the genera *Acaulospora* (4 spp.), *Entrophospora* (1 sp.), *Glomus* (7 spp.) and *Scutellospora* (2 spp.) were found. Among these, *Glomus* was the dominant genus found at all sites, followed by *Acaulospora*. The spore density and root colonization of AMF on chili did not vary significantly among the sites. The effects of ten selected AMF species on the growth of chili showed that *Gl. clarum* RA0305 increased the growth, flowering, and fruit production of chili, and also increased the P uptake significantly, compared to non-mycorrhizal plants. This fungus showed the highest potential as a promoter of growth, flowering and yield in organically managed chili production.

Keywords Chili growth · Diversity · *Glomus clarum* · Organically managed soil

S. Boonlue (✉)
Department of Microbiology, Faculty of Science,
Khon Kaen University, Khon Kaen 40002, Thailand
e-mail: bsopho@kku.ac.th

W. Surapat · C. Pukahuta
Department of Biological Sciences, Faculty of Science,
Ubon Ratchathani University,
Ubon Ratchathani 34190, Thailand

P. Suwanarit
Department of Microbiology, Faculty of Science,
Kasetsart University, Bangkok 10903, Thailand

A. Suwanarit
Department of Soil Science, Faculty of Agriculture,
Kasetsart University, Bangkok 10903, Thailand

T. Morinaga
Department of Environment Sciences, Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima,
Shoubara, Hiroshima 727-0023, Japan

Introduction

Chili (*Capsicum frutescens* L.) is an economically important vegetable in Thailand. In the northeast of Thailand, it is widely distributed in Ubon Ratchathani, Sisaket and Khon Kaen provinces. In general, there are two cultivation systems (conventional and organic) that are employed for chili production in Thailand. Most Thai chili farmers use conventional methods, applying chemicals such as synthetic fertilizers and synthetic pesticides, often in high doses. Because of the high chemical input, pesticide residues in the plant products may be harmful to consumers (Thapinta and Hudak 2000). For organically managed systems, green manure and animal manures, liquid "effective microorganisms" (so-called EM) and compost are applied. As a result, such chili products are free from pesticide residues and are safe for consumers. Accordingly, farmers receive higher prices for their products (Thapa and Rattanasuteerakul 2011).

Arbuscular mycorrhizal fungi (AMF), members of the phylum Glomeromycota, form symbiotic associations with the roots of more than 80% of land plants (Schussler et al. 2001; Gosling et al. 2006), including crop plant species.

Davies et al. (1992) reported that the roots of chili normally form a symbiotic association with AMF. In mycorrhizal associations, AMF have been shown to be beneficial to the host plant by increasing nutrient uptake, particularly phosphorus (P), as well as nitrogen (N), potassium (K), and micronutrients (Perner et al. 2007). In addition, AMF provide other benefits to the host plant, such as enhanced tolerance or resistance to soil pathogens and non-biotic stresses, and they also improve the soil structure (Smith and Read 1997). Because of this wide range of benefits to the host, AMF have received much interest for use in organic agricultural systems.

Organic farming is a form of agriculture that excludes most synthetic biocides and fertilizers (IFOAM 1998). Within the general principles of organic farming, AMF are usually considered to play an important role; it is assumed that they can compensate for the reduced use of fertilizers, and can be used as a good biocontrol agent against plant pathogens. Some reports have shown the effects of AMF on the colonization and growth of plants cultivated organically, but clear conclusions on this topic are yet to be drawn (Gosling et al. 2006).

There have been several reports on the effects of AMF on the growth of chili. The results have all shown high potential for enhancing the growth of chili fertilized with synthetic P fertilizers (Bagyaraj and Sreeramulu 1982; Davies et al. 2000; Martin and Stutz 2004). However, there have been no reports on the effects of AMF on chili cultivated organically. Therefore, the present study aimed to examine the status and diversity of AMF among chili plants cultivated under organic conditions, and to investigate the effects of AMF on the growth and nutrient uptake of chili (cv. Hua Rua) planted in organically managed soil under greenhouse conditions.

Materials and methods

Soil samples were collected from organic chili fields at four sites: Ban Hua Rua and Ratchathani Asok in Ubon Ratchathani province, and Ban Pone Yang and Ban Wang Hin in Sisaket province. At these sites, chili has been grown organically for 5–7 years by applying green manure and animal manures (the main sources of N) along with compost and EM to the soil. Rhizosphere soils (0–15 cm depth) and roots of chili were collected from chili plots in Ubon Ratchathani and Sisaket provinces during seasonal cultivation (June–August 2006). Samples of root-zone soils (each approximately 2 kg) surrounding chili plants, along with fine roots, were collected from the central areas (4 × 4 m) of three organic chili plots at each of the four sites, with duplicates from each plot. Each soil sample was air dried; AMF spores were then extracted while the roots were taken for assessment of AMF colonization.

Fresh roots of chili were processed by washing them free of soil. They were kept in 2.5% (w/v) KOH at 90°C in a water bath for 10–30 min, washed 5 times with tap water, soaked overnight with 1% (v/v) HCl, and stained with trypan blue in acetic glycerin solution (Koske and Gemma 1989). The percentage colonization of AMF in the roots of chili was then determined by the method described by Trouvelot et al. (1986). Using this technique, the stained roots were cut into short pieces (approximately 1 cm in length) and then placed on glass slides. Ten pieces of the stained root were put on each slide; each sample was prepared in triplicate. The AMF colonization on each root piece was estimated under a compound microscope using a 0–5 score numbering system:

Score 0 = no root colonization
Score 1 = <1% colonization
Score 2 = 1–10% colonization
Score 3 = 11–50% colonization
Score 4 = 51–90% colonization
Score 5 = >90% colonization

For each score, the number of root pieces that obtained that score was determined for each sample on triplicate slides (10 pieces per slide), and these numbers were then employed to calculating the percentage root colonization of the sample using the following equation:

$$\%M = (90n_5 + 70n_4 + 30n_3 + 5n_2 + n_1)/N,$$

where $\%M$ is the percentage root colonization, N is the total number of observed root pieces, while n_5, n_4,… and n_1 are the numbers of root pieces that obtained score numbers of 5, 4,… and 1, respectively.

AMF spores were extracted in triplicate from 5 g air-dried soil samples by a sucrose centrifugation method (Daniels and Skipper 1982). The supernatant containing AMF spores was poured onto a fine sieve with a pore diameter of 45 μm. The debris on the sieve was then washed with distilled water until the water flowing out of the sieve was clear. The spores were collected on grid-patterned (1 × 1 cm) filter paper and counted using a dissecting microscope.

AMF spores were separated from 100 g soil samples, which were randomly taken from each of the duplicate 2 kg soil samples from each plot, with two replications using wet sieving and decanting methods (Gerdemann and Nicolson 1963) through a stack of sieves with pore sizes of 250, 125, 90 and 45 μm in diameter. Spores were separated into groups according to general morphological similarities under an stereomicroscope (Olympus, SZ30). The spores were then surface sterilized by 0.2% chloramine-T for 5–10 min and washed 3 times with sterile distilled water.

Each spore morphotype was subjected to multiplication in pot cultures for further taxonomic identification and determination of chili growth promotion. Maize was used as the host plant for the pot cultures. AMF spores were inoculated on surface-sterilized maize seeds (10% sodium hypochlorite for 30 min) which were sown in plastic pots (12 in. diameter), 3 seeds per pot, containing twice-sterilized low-nutrient soil:loamy sand with a pH of 4.0, 0.8% organic matter, 0.04% total N, 20.0 ppm total P, 3.2 ppm available P (Bray II Method; Olsen and Dean 1965), 11.6 ppm extractable K (1 N NH_4OAc; Pratt 1965). The plants were grown in a greenhouse (30–35°C) with a transparent plastic roof and open sides. Deionized water prepared from chlorine-treated tap water was given to the plants via saucers until about 14 days after transplanting, and thereafter by spraying on the soil surface. Rats and ants were carefully controlled to prevent contamination of the soil in pots. No fertilizers or chemicals for pest control were applied to the soil. After tasselling, the plants were cut just above the soil surface and the soil was allowed to dry out in the pot. After drying, the soil was crushed by hand and used as an inoculum for the pot experiment described below. To ensure that the produced spores were of the specified species, spores from the produced inoculum sample were separated from the soil, as described above. The separated spores were mounted on glass slides in polyvinyl alcohol–lactoglycerol (PVLG) and PVLG + Melzer's reagent and then identified by species according to the INVAM AMF culture collection (http://www.invam.caf.wvu.edu) and the AMF identification manual by Schenck and Pérez (1988).

A pot experiment was carried out in a greenhouse employing a randomized complete block design with four replications and eleven treatments. The treatments consisted of one control (not inoculated with AMF) and ten AMF species: *Acaulospora foveata* Trappe & Janos HR0602, *A. appendicula* Sieverding & Schenck HR0201, *A. denticulata* Sieverding & Toro RA2106, *Glomus dimorphicum* Boyetchko & Tewari WH0101, *Gl. tenerum* Tandy WH0102, *Gl. clarum* RA0305, *A. denticulata* HR0406, *Gl. globiferum* Koske & Walker PY0109, *Gl. globiferum* PY0103, and *Gl. globiferum* PY0107. The soil used for the preparation of seedlings and this pot trial was obtained from an organically managed plot; it had a silt loam texture, a pH of 4.6, 1.3% organic matter, 0.08% total N, 66.8 ppm total P, 37.5 ppm available P, and 78.3 ppm extractable K. Chili (cv. Hua Rua) seedlings were prepared from sterile (10% sodium hypochlorite, 10 min) seeds planted in small plastic pots (top diameter, 7 cm) filled with autoclaved soil. After 14 days, individual chili seedlings were transplanted into individual plastic pots (top diameter, 12 in.) containing soil from Ratchathani Asok (a farm organically managed for more than 5 years) that had been fumigated with 60 g/m^2 of Dazomet (3,5-dimethyl-1,3,5-thiadiazinane-2-thione). The soil inocula of AMF prepared for each treatment consisted of spores, extraradical mycelium, and mycorrhizal roots. Approximately 100 spores of soil inoculum were placed in the bottom of a central hole in a pot before transplanting. The plants were watered with deionized water, as described for inoculum production, for 90 days. No fertilizers or pesticides were applied. At the end of the study, plant growth parameters were determined, including shoot height, stem diameter, fresh weights of shoots and roots, dry weights of shoots and roots, number of flowers per plant, number of fruits per plant, and major plant nutrient uptake (N, P, K). In addition, the percentage of root colonization and the spore density of AMF were also examined.

The data were analyzed using SAS statistical software. All data were subjected to analysis of variance. Comparisons of means were made by Duncan's multiple range test ($P \leq 0.05$).

Results and discussion

Colonization and spore density of AMF in organically grown chili

As shown in Table 1, the root colonization and spore density of AMF did not differ among the studied sites. This might be due to the similar agricultural practices of the farmers (applying green manure, animal manures, EM and compost). Generally, the communities and colonization of AMF in organically managed soil are strongly influenced by management techniques, such as the utilization of fertilizers (e.g., green manure, compost, animal manures), crop plant species, and cultivation systems. In addition, AMF species show great diversity depending on habitat, functional interactions with their host (Bending et al. 2004), and variations in the host species within the natural ecosystem (Sieverding 1989).

Fungal taxa

Fourteen spore monotypes were identified in the field soil (Table 1). The spores were classified into the following genera: *Acaulospora* (4 spp.), *Entrophospora* (1 sp.), *Glomus* (7 spp.) and *Scutellospora* (2 spp.). AMF belonging to the genus *Glomus* were observed to be dominant in the rhizosphere soil of chili at all sites. In addition, the species *Gl. globiferum* could be observed in all sites, while *A. appendicula* and *S. heterogama* were distributed in three and two sites, respectively. The finding of *Glomus* spp. as the dominant genus was in agreement with the results found in the 22-year organic agricultural

Table 1 Root colonization in chili (*Capsicum frutescens* L.), spore density and species of AMF in soils from organic chili plots during seasonal cultivation ($n = 2$)

Sampling sites	% AMF colonization[a]	Spore density per 1 g soil[a]	AMF species or spore description	Site latitude and longitude
Ban Hua Rua, Muang amphur, Ubon Ratchathani province	59.2 ± 11.3 a	3.5 ± 2.6 a	*Acaulospora appendicula* Spain, Sieverding & Schenck	15°22′4.82″N
			Acaulospora denticulata Sieverding & Toro	104°49′49.74″E
			Acaulospora foveata Trappe & Janos	
			Acaulospora scrobiculata Trappe	
			Glomus etunicatum Becker & Gerdemann	
			Glomus globiferum Koske & Walker	
			Glomus sp. 1: ellipsoidal to oval shape, 63–88 μm, white to cream color, shiny and containing globose lipid content, single chlamydospore. One spore wall, irregular pitted wall. Hyphal attachment wall combined to spore wall, hyaline color	
			Glomus sp. 2: globose to oval shape, red-brown to dark brown sporocarp formation with 148–260 μm peridium tightly enclosing sporocarp, yellow-brown to red-brown colored chlamydospores (11–16 × 17–28 μm)	
			Scutellospora heterogama (Nicol. & Gerd.) Walker & Sanders	
Ratchathani Asok, Warin Chamrap amphur, Ubon Ratchathani province	45.7 ± 19.2 a	2.7 ± 1.6 a	*Acaulospora appendicula* Spain, Sieverding & Schenck	15°13′34.48″N
			Acaulospora foveata Trappe & Janos	104°54′11.05″E
			Glomus clarum Nicolson & Schenck	
			Glomus dimorphicum Boyetchko & Tewari	
			Glomus globiferum Koske & Walker	
			Glomus leptotichum Schenck & Smith	
			Entrophospora infrequens (Hall) Ames & Schneider	
Ban Pone Yang, Wang Hin amphur, Sisaket province	52.3 ± 9.3 a	7.9 ± 5.7 a	*Glomus globiferum* Koske & Walker	14°56′9.16″N
			Glomus etunicatum Becker & Gerdemann	104°12′4.23″E
			Glomus sp. 2: globose, oval to irregular shape, red-brown to dark-brown sporocarp formation with 148–260 μm peridium tightly enclosing sporocarp, yellow-brown to red-brown color chlamydospores (11–16 × 17–28 μm)	
			Scutellospora sp.: globose shape, 279–340 μm, golden yellow to orange-brown color, single chlamydospore. Two groups of spore walls consisting of three wall layers	
			Group one: brown to dark brown colored outer layer, unit wall type, thickness 4 μm, combined to the laminated wall of the second layer, thickness 7–8 μm	
			Group two: membranous wall type, thickness 3–4 μm. Bulbous suspensor; globose, 50–70 μm, golden yellow color, wall thickness 3–4 μm.	
Ban Wang Hin, Wang Hin amphur, Sisaket province	50.1 ± 9.6 a	4.9 ± 5.3 a	*Acaulospora appendicula* Spain, Sieverding & Schenck	14°55′4.58″N
			Glomus globiferum Koske & Walker	104°14′31.66″E
			Scutellospora heterogama (Nicol. & Gerd.) Walker & Sanders	
CV (%)	9.4	41.7		

In a column, means followed by a common letter are not significantly different by $DMRT_{0.05}$

[a] Mean of three field plot replicates ± standard deviation

system reported by Oehl et al. (2004); in maize, by Na Bhadalung et al. (2005); and in soybean by Franke-Snyder et al. (2001). Muthukumar et al. (2003) reported that *Glomus* (93%) was more dominant than *Acaulospora* (53%), *Gigaspora* (23%) and *Scutellospora* (18%) in their study. Muthukumar and Udaiyan (1999), Zhao et al. (2001),

and Chubo et al. (2009) documented that *Glomus* and *Acaulospora* are more dominant in tropical soil than other mycorrhiza genera. In a study by Chubo et al. (2009), *Glomus* and *Acaulospora* (respectively) were found to be the dominant genera. Similar results were also found in a study of AMF associated with the Meliaceae group on Hainan Island, China (Shi et al. 2006). Ananthakrishnan et al. (2004) documented that the ability of *Glomus* to dominate the soil rhizosphere indicated that *Glomus* has a broad host range and is able to thrive in a wide variety of environmental conditions as compared to other AMF genera.

Effects of AMF on the growth of chili

Effects of AMF inoculation on the growth and nutrient uptake of chili plants are shown in Tables 2 and 3. Plants inoculated with *A. appendicula* HR0201 and *A. denticulata* RA2106 showed significantly higher values than uninoculated plants (control) for all plant parameters except height and number of fruits (Table 2). *Gl. clarum* RA0305 demonstrated the greatest ability to increase most growth parameters, including height, fresh and dry weight of shoots, fresh weight of roots, and number of fruits per plant, and showed the best trends in terms of increasing stem diameter, dry weight of roots, and number of flowers per plant.

It was found in our research that only *Gl. clarum* RA0305 contributed to P uptake (Table 3) in chili, causing higher total amounts of P per pot than any other AMF treatment. In addition, only the plants inoculated with *A. denticulata* HR0406 were found to have significantly higher N and K uptake than uninoculated plants. Our findings correspond to results reported by Perner et al. (2007), who found that P and K uptake in pelargonium (*Pelargonium peltatum*) was enhanced by AMF. They found low P and K concentrations in shoots of uninoculated plants, whereas plants treated with AMF had high P concentrations and adequate K concentrations. N concentrations in pelargonium shoots were not significantly different between uninoculated and inoculated plants. Although mycorrhizal fungi are well known for their efficient P uptake, particularly in P-deficient soil, the contribution of K to plants by AMF has rarely been described, specifically in regard to acid soil (Perner et al. 2007). In terms of percent root colonization, *Gl. clarum* RA0305 gave the highest figures. However, AMF spore density in all plants inoculated with AMF did not vary significantly. The growth parameters and P uptake of chili plants in the present study suggested that *Gl. clarum* RA0305 had the highest potential for promoting chili growth, as a result of its highest potential for enhancing P uptake of the plant. Accordingly, it should be worth

Table 2 Growth parameters and root colonization in chili and spore density in soils inoculated with different AMF species

Treatments	Shoot				Root		Number of flowers per plant	Number of fruits per plant	Percentage of root colonization	Spore density per 1 g soil
	Height (cm)	Stem diameter (cm)	Fresh weight (g/pot)	Dry weight (g/pot)	Fresh weight (g/pot)	Dry weight (g/pot)				
Control	41.8 a	0.38 a	10.2 a	1.5 a	12.0 a	6.1 a	0.0 b	0.0 b	0.0 c	0.0 b
Acaulospora foveata HR0602	38.0 a	0.33 a	6.8 a	1.1 a	10.1 a	5.5 a	1.5 b	0.0 b	50.0 b	4.3 a
Acaulospora appendicula HR0201	61.0 a	0.56 b	36.5 b	5.3 b	47.6 b	21.9 b	35.0 a	1.8 b	52.4 b	5.0 a
Acaulospora denticulata RA2106	59.3 a	0.56 b	50.0 b	3.7 b	35.1 b	21.4 b	37.0 a	4.5 b	60.3 ab	5.4 a
Glomus dimorphicum WH0101	44.3 a	0.39 a	14.4 a	2.4 a	20.8 a	6.2 a	4.8 b	1.8 b	38.5 b	5.4 a
Glomus tenerum WH0102	31.8 a	0.30 a	4.8 a	0.8 a	8.8 a	4.2 a	0.3 b	0.0 b	49.5 b	5.6 a
Glomus clarum RA0305	71.5 b	0.62 b	68.8 c	11.4 c	53.2 c	26.5 b	53.0 a	9.5 a	79.7 a	6.5 a
Acaulospora denticulata HR0406	22.5 a	0.26 a	2.4 a	0.4 a	6.0 a	3.4 a	0.0 b	0.0 b	56.9 ab	5.3 a
Glomus globiferum PY0109	36.5 a	0.37 a	11.5 a	2.2 a	20.5 a	9.7 a	11.5 b	2.0 b	50.5 b	4.7 a
Glomus globiferum PY0103	43.5 a	0.34 a	9.9 a	1.5 a	11.3 a	6.5 a	2.0 b	0.3 b	58.5 ab	3.9 a
Glomus globiferum PY0107	35.3 a	0.29 a	4.7 a	0.8 a	8.1 a	3.4 a	1.0 b	0.0 b	57.8 ab	4.0 a
CV (%)	62.9	20.5	75.0	65.3	54.7	53.8	107.0	161.7	25.6	56.6

In a column, means followed by a common letter are not significantly different by $DMRT_{0.05}$

Table 3 N, P and K uptake of chili plants as affected by different AMF

Treatments	N uptake (mg per pot)	P uptake (mg per pot)	K uptake (mg per pot)
Control	0.203 a	0.010 a	0.307 a
Acaulospora foveata HR0602	0.296 a	0.007 a	0.460 a
Acaulospora appendicula HR0201	0.217 a	0.011 ab	0.392 a
Acaulospora denticulata RA2106	0.498 ab	0.023 ab	0.848 a
Glomus dimorphicum WH0101	0.372 a	0.011 ab	0.618 a
Glomus tenerum WH0102	0.602 ab	0.013 ab	1.131 a
Glomus clarum RA0305	0.594 ab	0.031 b	0.952 a
Acaulospora denticulata HR0406	1.083 b	0.024 ab	2.768 b
Glomus globiferum PY0109	0.474 ab	0.009 ab	1.178 a
Glomus globiferum PY0103	0.214 a	0.008 a	0.421 a
Glomus globiferum PY0107	0.524 ab	0.010 ab	1.022 a
CV (%)	55.3	56.2	75.1

In a column, means followed by a common letter are not significantly different by $DMRT_{0.05}$

investigating its effect when introduced as a biofertilizer to actual organic chili cropping. Our results correspond with those of Kahiluoto and Vestberg (1998), who found that AMF in organically managed soil were effective at increasing growth and P uptake in crop plants. However, the authors reported that crop yields were not always greater, although P use efficiency increased. In the present study, three AMF—*A. appendicula* HR0201, *A. denticulata* RA2106 and *Gl. clarum* RA0305—were found to be good promoters of chili growth in sterile, organically managed soil, although the available P content in the soil used in this study was quite high (37.5 ppm). This implies that these three mycorrhizal fungi may be insensitive to high available P in soil, as was found by Na Bhadalung et al. (2005). Our results indicated that these three AMF isolates were efficient and might be suitable for use in organic agricultural fields. However, some data have indicated that using AMF for cultivated plants in an organic system may not be successful (Scullion et al. 1998). This may be a result of management practices unfavorable to AMF (Gosling et al. 2006). Therefore, further studies are needed before using the fungi in the field.

Regarding flowering and the number of fruits, *A. appendicula* HR0201, *A. denticulata* RA2106 and *Gl. clarum* RA0305 increased the number of flowers per plant, whereas only the latter AMF increased the number of fruits per plant. *Gl. clarum* RA0305 was the best at enhancing these two parameters. Bagyaraj and Sreeramulu (1982) reported that chili inoculated with mycorrhizal fungi had more flowers and a higher yield of green fruits compared with uninoculated chili. Koide (2000) reported that *Abutilon theophrasti* infected by mycorrhizal fungi had significantly increased numbers of flowers and fruits per plant compared to non-mycorrhizal plants. These results were similar to those from soybean (*Glycine max*) (Busse and Ellis 1985; Schenck and Smith 1982) as well as pelargonium (Perner et al. 2007). In addition, it was also observed in the present study that chili inoculated with *Gl. clarum* RA0305 flowered earlier and produced more flowers than other AMF (data not shown). Koide (2000) documented that the time taken to initiate flowering decreased when plants grown in P-deficient soil were inoculated with mycorrhizal fungi. This resulted in lengthened flowering duration and an increased number of flowers produced per plant. Moreover, Bagyaraj and Sreeramulu (1982) reported that plant flowering was controlled by hormones. Perner et al. (2007) documented that mycorrhizal colonization may either directly influence plant hormonal balance or may indirectly affect plant hormone levels by altering plant nutrient status. Therefore, further study is needed for clarification.

Conclusions

The results of this study showed that 14 AMF taxa were identified in the study areas: *Acaulospora* (4 spp.), *Entrophospora* (1 sp.), *Glomus* (7 spp.) and *Scutellospora* (2 spp.). *Glomus* was the dominant genus, and was found in all sites of organically grown chili. The spore density and root colonization of organic chili did not vary significantly, due to the similar agricultural practices of the farmers. *Acaulospora appendicula* HR0201, *A. denticulata* RA2106 and *Gl. clarum* RA0305 were found to be efficient chili growth promoters, with *Gl. clarum* RA0305 being the best. They not only increased growth but also enhanced flowering and fruiting. The latter AMF also increased the P uptake. Additionally, these isolates were insensitive to high soil P status. These findings suggest the potential of *Gl. clarum* RA0305 for use as an AMF inoculum for the production of organic chili in Thailand.

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