



Original article

Comparative potentials of native arbuscular mycorrhizal fungi to improve nutrient uptake and biomass of *Sorghum bicolor* LinnPattarawadee Sumthong Nakmee,^{a,*} Sombun Techapinyawat,^b Supranee Ngamprasit^c^a Faculty of Science at Sriracha, Kasetsart University, Sriracha Campus, Chonburi 20230, Thailand^b Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand^c The National Corn and Sorghum Research Center, Nakhon Ratchasima Province, Kasetsart University, Nakhon Ratchasima 30320, Thailand

ARTICLE INFO

Article history:

Received 1 May 2015

Accepted 31 January 2016

Available online 25 June 2016

Keywords:

Arbuscular mycorrhiza

Sorghum

Nutrient uptake

Biomass

ABSTRACT

Sorghum (*Sorghum bicolor* Linn.) seedlings were grown in pots using Pakchong soil from Nakhon Ratchasima province. Ten species of native Arbuscular mycorrhizal (AM) fungi: *Glomus* sp. 1, *Glomus* sp. 2, *Glomus* sp. 3, *Glomus aggregatum*, *Glomus fasciculatum*, *Acaulospora longula*, *Glomus occultum*, *Acaulospora scrobiculata*, *Acaulospora spinosa* and *Scutellospora* sp., were used to inoculate sorghum seedlings. The sorghum growth and uptake of several major nutrients were evaluated at the harvesting stage. The results revealed that sorghum inoculated with *A. scrobiculata* produced the greatest biomass, grain dry weight and total nitrogen uptake in shoots. The highest phosphorus uptake in shoots was found in *A. spinosa*-inoculated plants, followed by *Glomus* sp. and *A. scrobiculata*, whereas *Scutellospora* sp.-inoculated plants showed the highest potassium uptake in shoots followed by *A. scrobiculata*. Overall, the most efficient AM fungi for improvement of nutrient uptake, biomass and grain dry weight in sorghum were *A. scrobiculata*.

Copyright © 2016, Kasetsart University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Arbuscular mycorrhizal (AM) fungi are among the most ubiquitous soil microorganisms, forming mutualistic associations with 80–90% of vascular plant species in ecosystems throughout the world (Harrison, 1997; Smith and Read, 1997). Previous research has indicated that inoculation with AM fungi enhances growth and the nutrient uptake of phosphorus (McArthur and Knowles, 1993) and nitrogen (Barea et al., 1987; Azco'n Aguilar et al., 1993). Inoculation with AM fungi increased the percentage of nitrogen uptake and fruit yield of green peppers in high P soil (Douds and Reider, 2003), and promoted plant biomass and enhanced P, K, Ca, Fe, Mn and Cu uptake in chickpea plants in pot experiments using soil with high levels of available P and K (Farzaneh et al., 2011). Moreover, AM fungi increased the activities of soil enzymes such as phosphatase which can degrade organic phosphate (i.e. phytate) to available phosphate (Dodd et al., 1987; Kothari et al., 1990; Vázquez et al., 2000). Oxalic acid released from AM fungi reacts with unavailable phosphate, converting it into available phosphate (Beever and Burns, 1980). However, in addition, AM fungal mycelia

effectively increased the total absorption surface of inoculated plants and thus improved plant access to nutrients such as P, Cu and Zn (Lambert et al., 1979; George et al., 1994; Ortas et al., 1996). AM fungi extended the absorbing network beyond the nutrient-depletion zones of the rhizosphere which allowed access to a larger volume of soil than for roots not colonized by AM fungal mycelium. There is additional evidence showing that AM fungi helped plants to acquire nutrients including P, Zn, N, Cu and K (Marschner and Dell, 1994; Cavagnaro, 2008; Lehmann et al., 2014). Research by Cavagnaro et al. (2015) showed that AM fungi had the ability to reduce nutrient loss from the soil by enlarging the nutrient interception zone and preventing nutrient loss after rain-induced leaching events.

Many reports found that plants hosting AM fungi symbiosis in root systems are tolerant to drought and plant pathogenic microorganisms (Bethlenfalvay and Linderman, 1992; Tobar et al., 1994; Subramanian et al., 1995). Augé (2001) found that AM fungi enhanced water relations and improved the soil structure (Miller and Jastrow, 2000). Thus effective utilization of AM fungal symbiosis should benefit crop production systems, pest control and alternatives to chemical fertilizers.

ICRISAT (2009) summarized some global statistics on sorghum. It is the fifth most important cereal crop after wheat, rice, maize and barley and is the dietary staple of more than 500 million

* Corresponding author.

E-mail address: pattarawadee@src.ku.ac.th (P.S. Nakmee).

people in more than 30 countries around the world. It is grown on 42 million ha in 98 countries of Africa, Asia, Oceania and the Americas. Approximately 55% of sorghum grain is used for food purposes and 33% is used as feed grain in the Americas. Most farmers grow sorghum as the second crop at the end of rainy season, after maize.

AM fungal root colonization in host plants is nonspecific and more than one species of AM fungi have been found across multiple plant species (Boyetchko and Tewari, 1990; Simpson and Daft, 1990; Tewari et al., 1993). The potential of native AM fungi that improve the growth and nutrient uptake of crop plants such as sorghum is therefore of great interest. Effective AM fungi inocula might be an alternative source for sorghum biofertilizer and for sustainable agriculture. The objective of this experiment was to find suitable AM fungal species that can improve the production of sorghum.

Materials and methods

Collection of arbuscular mycorrhizal fungi

The AM fungi were collected from 20 soil samples of sorghum rhizosphere in Lopburi, Nakorn Ratchasima, Pitsanulok and Saraburi provinces in Thailand. AM fungal spores were isolated from soil samples using a wet sieving and decanting method (Gerdemann and Nicolson, 1963). Morphological characteristics analysis and identification of AM fungi followed the Manual for the Identification of VA Mycorrhizal Fungi (Schenck and Pérez, 1988) in collaboration with the Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand. The highest spore numbers of the same species from each soil sample were propagated in corn-seedling pot culture to produce AM fungal inocula. Corn grains were surface sterilized with 10% Clorox. Pakchong soil was collected from the National Corn and Sorghum Research Center, Nakhon Ratchasima province and soil was sterilized with dazomet for 30 d before being used in the pot culture. The successful inocula of each AM fungal species were sampled to evaluate the spore density by counting the number of AM fungal spores per gram of soil using the sucrose centrifugation method (Jenkins, 1964).

Pot experiment

Sorghum grains (variety KU 439) were surface sterilized with 10% Clorox. Pakchong soil was collected from the National Corn and Sorghum Research Center, Nakhon Ratchasima province and sterilized with dazomet for 30 d. Ten kg of soil was added into each of 44 plastic pots (30 cm in diameter). The soil pH was 6.9, organic matter was 4.0%, total nitrogen was 0.183% (Kjeldahl method), available phosphorus was 12 ppm (Bray II method), potassium was 115 meq/100 g, calcium was 17 meq/100 g, magnesium was 2 meq/100 g (atomic absorption spectrophotometer, Unicam 929; Unicam; Leeds, UK) and electrical conductivity was 0.12 mΩ at 25 °C using a conductivity probe (YSI-85, YSI; Yellow Springs, OH, USA). Ten sorghum grains were grown in each pot for 5 d and then two uniform seedlings from each pot were selected for this experiment. Inocula of AM fungi were placed into each pot at the sorghum seedling rhizosphere. A completely randomized design was used in this experiment with 11 experimental treatments: one control (T1) and 10 AM fungal species inoculations (T2–T11), with four replications each. The statistical analysis used the IRRISTAT program (version 4.0 for Windows; IRRISTAT; Manila, the Philippines) with Duncan's Multiple's Range Test.

Harvest and analysis

The sorghum plants were harvested 120 d after AM fungal inoculation. The plant height, number of leaves, biomass, spore density (Jenkins, 1964) and percentage of AM fungal root colonization (Trouvelot et al., 1986) were evaluated. The flowering period was observed during plant growth. The plant dry weight was measured separately into shoots, roots and grains after harvesting. Dry sorghum shoots and roots were used to analyze the phosphorus uptake using the Vanado molybdate method, the total nitrogen uptake using the Kjeldahl method and the potassium uptake using atomic absorption spectrophotometry (Chapman and Pratt, 1978).

Results and discussion

Morphological characteristics of AM fungi

Fungal identification and description of morphological characteristics of *Glomus aggregatum*, *Glomus fasciculatum*, *Glomus occultatum*, *Acaulospora longula*, *Acaulospora scrobiculata*, *Acaulospora spinosa*, *Glomus* sp. 1, *Glomus* sp. 2, *Glomus* sp. 3 and *Scutellospora* sp. followed the Manual for the Identification of VA Mycorrhizal Fungi (Schenck and Pérez, 1988). The morphological characteristics of four unknown species were described as follows.

Glomus sp. 1: Chlamydo spores formed in sporocarps without a peridium. Light yellow globose spore with 53.97–74.53 μm diameter. Spore wall 1.29–2.57 μm thick and consisting of two layers, each layer light yellow in color, membranous, 1 μm thick. Hyphal attachment straight or recurved. Hyphae with slight swelling at the point of spore attachment.

Glomus sp. 2: Chlamydo spores form singly in the soil, globose, 157.8–199.8 μm diameter. Spore wall brown in color, 7.71–10.28 μm thick and consisting of two layers. Outer wall laminate and brown in color, frequently with soil particles or debris adhering, 5.14–5.26 μm thick. Inner wall membranous and yellow-brown in color, 1 μm thick. Hyphal attachment straight or recurved.

Glomus sp. 3: Chlamydo spores form singly in the soil, yellow to yellow-brown or orange-brown, globose to irregular, 92.4–147.3 μm diameter. Spore wall consisting of three walls in one group. Wall 1 (outer wall): a yellow-brown to orange-brown unit wall, 4.63–5.14 μm thick, irregular blister-like areas on the outer surface. Wall 2: pale yellow to yellow-brown, laminate and rough, 3.85–5.14 μm thick. Wall 3: a membranous wall. Hyphal attachment straight, recurved or funnel.

Scutellospora sp.: Azygospores form free in soil, borne end of hyphae on light brown bulbous suspensor-like cell. Spore globose to subglobose, 284–377 μm diameter with light yellow to light greenish yellow color. Spore wall consisting of three layers. Outer wall laminate and light greenish yellow but frequently with soil particles or debris adhering, 5.14–7.71 μm thick. Wall 2: laminate and rough, 3.84–5.14 μm thick. Wall 3: laminate, 1.28–2.57 μm thick.

Growth and biomass

The height of sorghum after harvesting (at 120 d) of all experimental treatments was between 82.70 and 95.80 cm. AM fungal inoculation did not greatly affect the sorghum height because the sorghum height was genetically controlled (Hadley, 1957). AM fungal inoculation significantly increased the number of leaves. The sorghum inoculated with *Glomus* sp. 3, *G. fasciculatum* and *A. scrobiculata* had the highest number of leaves (10.30 leaves/plant), while the control had the lowest number of leaves (8.10 leaves/plant). The sorghum inoculated with *Glomus* sp. 1 had the

shortest flowering period (62.80 d) compared with sorghum inoculated with other AM fungal species and the control (68.00 d). The highest number of AM fungal spores in the soil was found with the *A. scrobiculata* inoculation (14.10 spores/g soil). The highest percentage of AM fungal root colonization was found in sorghum inoculated with *A. spinosa* (23.58%), followed by *Glomus* sp. 1 (22.88%) and *A. scrobiculata* (21.63%) respectively. The results are shown in Table 1.

AM fungal inoculation significantly increased the growth and biomass of sorghum with enhanced shoot, root and grain dry weight when compared with the control. Sorghum inoculated with *Scutellospora* sp. produced the highest shoot dry weight (34.34 g/plant), while the root dry weight of sorghum inoculated with *Glomus* sp. 2, *A. longula* and *A. scrobiculata* was 7.92 g/plant, 7.91 g/plant and 7.77 g/plant, respectively, higher than the other AM fungal inoculations. Sorghum inoculated with *A. scrobiculata* had the greatest plant biomass and grain dry weight with values of 57.24 and 16.05 g/plant respectively. The results are shown in Table 2. These results correspond with those reported by Ortas et al. (1996) who found that AM fungi enhanced the growth of sorghum.

Nutrient uptake

AM fungal inoculation significantly increased the percentage of nitrogen in shoots and the total nitrogen uptake in shoots and roots of sorghum as measured after harvesting. AM fungi increased the percentage and the total nitrogen uptake in sorghum shoots compared with the control. *A. scrobiculata* inoculation had the highest percentage of nitrogen (1.01%) and total nitrogen uptake (33.65 mg/plant) in shoots. The percentages of nitrogen in roots were not significantly different across treatment inocula, though *A. longula* inoculation resulted in the highest total nitrogen uptake in roots compared to the other treatments (Table 3).

The percentage of phosphorus and the available phosphorus uptake in the shoots and roots of sorghum as measured after harvesting were significantly increased by AM fungal inoculation. *Glomus* sp. 1 inoculation produced the highest percentage of phosphorus uptake in shoots (0.44%). However, *Glomus* sp. 2, *A. longula* and *Glomus occultum* inoculation did not increase the percentage of phosphorus in shoots compared to the control. Sorghum inoculated with *G. fasciculatum* resulted in the highest

percentage of phosphorus in roots (0.20%) compared with the other treatments (Table 4). *A. spinosa* and *Glomus* sp. 1 inoculation produced the highest phosphorus uptake in shoots with values of 14.50 mg/plant and 13.95 mg/plant, respectively. *A. longula* inoculation had the highest phosphorus uptake in roots (1.50 mg/plant) compared with other treatments (Table 4).

Significant increases in the percentage of potassium in shoots and potassium uptake in shoots and roots of sorghum were found following AM fungal inoculation compared with the control. *A. longula* inoculation had the highest percentage of potassium in shoots (1.38%). Sorghum inoculated with *Scutellospora* sp. had the highest potassium uptake in shoots (45.34 mg/plant) and *Glomus* sp. 2 inoculation had the highest potassium uptake in roots (10.30 mg/plant) compared to the other treatments (Table 5).

These results indicate that inoculation with AM fungal species significantly improved the nutrient uptake, growth and biomass in sorghum when compared with the control for the 10 species tested, *A. scrobiculata* was a suitable AM fungi and was the most effective at improving sorghum growth and biomass. This corresponded to Techapinyawat et al. (2000) who found that *A. scrobiculata* inoculation improved the growth and nitrogen, phosphorus and potassium uptake in *Vetiveria zizanioides*. The benefits of sorghum grown with mixed species of AM fungi (Ortas et al., 1996; Lenzemo and Kuypers, 2001) or *Glomus* spp. have been described for both growth and nutrient uptake but not when grown with *Acaulospora* sp. (Raju et al., 1990; Medeiros et al., 1994; Bagayoko et al., 2000; Guo et al., 2013).

High percentages of sorghum root colonization were found following *A. spinosa*, *Glomus* sp. 1 and *A. scrobiculata* inoculation. The highest phosphorus uptake in sorghum shoots was found with plants treated with *A. spinosa* (14.5 mg/plant), *Glomus* sp. 1 (14.0 mg/plant) and *A. scrobiculata* (13.0 mg/plant), respectively. The percentage of sorghum root colonization by *Glomus* sp. 1 and *A. scrobiculata* seemed to be correlated with nutrient uptake in sorghum. Allen et al. (1981) and Cress et al. (1979) reported that AM fungal root colonization improved the phosphorus uptake, phosphorus transfer, phosphorus activity and the use of available phosphorus in plants, which enhanced plant growth and biomass. Sorghum root colonization by AM fungi (other than *A. scrobiculata*) increased the growth, nutrient uptake and efficiency of water utilization (Pearson and Tinker, 1975; Raju et al., 1990; Simpson and

Table 1

Number of spores, percentage of root colonization, plant height, number of leaves and flowering period of *Sorghum bicolor* Linn. (KU 439).

Treatment	Number of spores (spores/g soil)	Root colonization (%)	Plant height (cm)	Number of leaves per plant	Flowering period (d)
Control	1.90 ^{e/1}	0.77 ^{d/1}	82.70 ^{b/1}	8.10 ^{d/1}	68.00 ^{a/1}
<i>Glomus</i> sp. 1	13.60 ^{ab}	22.88 ^a	95.80 ^a	9.40 ^{abc}	62.80 ^c
<i>Glomus</i> sp. 2	11.50 ^{bc}	16.42 ^b	91.20 ^{ab}	8.80 ^{bcd}	67.30 ^{ab}
<i>Glomus</i> sp. 3	7.80 ^d	11.74 ^c	88.80 ^{ab}	10.30 ^a	67.30 ^{ab}
<i>G. aggregatum</i>	12.80 ^{ab}	20.19 ^{ab}	94.30 ^a	9.60 ^{ab}	64.00 ^{bc}
<i>G. fasciculatum</i>	10.00 ^c	16.34 ^b	90.50 ^{ab}	10.30 ^a	65.80 ^{ab}
<i>A. longula</i>	11.70 ^{bc}	19.59 ^{ab}	91.00 ^{ab}	9.00 ^{bcd}	66.50 ^{ab}
<i>G. occultum</i>	12.00 ^{abc}	20.70 ^{ab}	90.00 ^{ab}	8.50 ^{cd}	65.80 ^{ab}
<i>A. scrobiculata</i>	14.10 ^a	21.63 ^a	93.50 ^{ab}	10.30 ^a	64.00 ^{bc}
<i>A. spinosa</i>	13.20 ^{ab}	23.58 ^a	92.00 ^{ab}	10.00 ^a	65.50 ^{abc}
<i>Scutellospora</i> sp.	12.50 ^{ab}	20.08 ^{ab}	91.10 ^{ab}	9.60 ^{ab}	66.60 ^{ab}
CV	13.00	16.00	7.20	6.30	2.80
LSD _{0.05}	2.04	4.03	9.47	0.86	2.66
LSD _{0.01}	2.75	5.42	12.72	6.33	3.57
F-test	28.15 ^{**}	22.39 ^{**}	1.80 ^{ns}	6.33 ^{**}	2.74 [*]

^{/1} in each column, mean (N = 4) followed by the different lower case letter in the same column are significantly different (p ≤ 0.05).

^{*}Significant difference (p ≤ 0.05).

^{**}Significant difference (p ≤ 0.01).

^{ns}Not significantly different.

CV = coefficient of variation.

LSD_{0.05} = Least Significant Difference at α = 0.05.

LSD_{0.01} = Least Significant Difference at α = 0.01.

Table 2
Shoot dry weight, root dry weight, grain dry weight and biomass of *Sorghum bicolor* Linn. (KU 439).

Treatment	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Grain dry weight (g/plant)	Biomass (g/plant)
Control	25.04 ^{e/1}	5.50 ^{d/1}	13.99 ^{bc/1}	44.53 ^{e/1}
<i>Glomus</i> sp. 1	31.73 ^{abc}	6.48 ^{bcd}	15.99 ^a	54.20 ^{abc}
<i>Glomus</i> sp. 2	27.99 ^d	7.92 ^a	15.44 ^a	51.35 ^{cd}
<i>Glomus</i> sp. 3	28.78 ^d	5.84 ^{cd}	13.78 ^c	48.40 ^d
<i>G. aggregatum</i>	32.78 ^{abc}	7.48 ^{ab}	15.50 ^a	55.76 ^{ab}
<i>G. fasciculatum</i>	30.64 ^{bcd}	6.35 ^{abc}	15.09 ^{ab}	52.08 ^{bcd}
<i>A. longula</i>	29.81 ^{cd}	7.91 ^a	13.76 ^c	51.48 ^{cd}
<i>G. occultum</i>	30.57 ^{bcd}	6.06 ^{bc}	15.15 ^{ab}	51.78 ^{cd}
<i>A. scrobiculata</i>	33.42 ^{ab}	7.77 ^a	16.05 ^a	57.24 ^a
<i>A. spinosa</i>	32.59 ^{abc}	6.72 ^{a-d}	15.48 ^a	54.79 ^{abc}
<i>Scutellospora</i> sp.	34.34 ^a	6.93 ^{abc}	15.26 ^{ab}	56.53 ^{ab}
CV	5.80	11.30	65.50	4.60
LSD _{0.05}	2.56	1.10	1.18	3.51
LSD _{0.01}	3.43	1.48	1.59	4.72
F-test	9.04 ^{**}	5.01 ^{**}	4.06 ^{**}	7.92 ^{**}

^{1/1} in each column, mean ($N = 4$) followed by the different lower case letter in the same column are significantly different ($p \leq 0.05$).

^{**}Significant difference ($p \leq 0.01$).

CV = coefficient of variation.

LSD_{0.05} = Least Significant Difference at $\alpha = 0.05$.

LSD_{0.01} = Least Significant Difference at $\alpha = 0.01$.

Table 3
Percentage of nitrogen and the total nitrogen uptake in *Sorghum bicolor* Linn. (KU 439).

Treatment	% Nitrogen		Total nitrogen uptake (mg/plant)	
	Shoots	Roots	Shoots	Roots
Control	0.70 ^{d/1}	0.35	17.51 ^{e/1}	1.90 ^{e/1}
<i>Glomus</i> sp. 1	0.95 ^{ab}	0.43	30.23 ^{ab}	2.81 ^{bcd}
<i>Glomus</i> sp. 2	0.68 ^d	0.49	19.00 ^{de}	3.26 ^{ab}
<i>Glomus</i> sp. 3	0.86 ^{a-b}	0.39	24.80 ^{cde}	2.24 ^{de}
<i>G. aggregatum</i>	0.97 ^{ab}	0.43	31.05 ^{ab}	3.17 ^{ab}
<i>G. fasciculatum</i>	0.86 ^{a-d}	0.38	26.20 ^{bc}	2.43 ^{de}
<i>A. longula</i>	0.75 ^{bcd}	0.44	22.39 ^{cde}	3.51 ^a
<i>G. occultum</i>	0.81 ^{bcd}	0.42	24.73 ^{abc}	2.53 ^{cd}
<i>A. scrobiculata</i>	1.01 ^a	0.40	33.65 ^a	3.12 ^{ab}
<i>A. spinosa</i>	0.90 ^{abc}	0.49	29.46 ^{ab}	3.32 ^{ab}
<i>Scutellospora</i> sp.	0.79 ^{bcd}	0.44	27.22 ^{bc}	3.05 ^{abc}
CV	13.60	13.70	15.20	12.90
LSD _{0.05}	0.17	0.08	5.70	0.52
LSD _{0.01}	0.22	0.11	7.66	0.71
F-test	3.55 ^{**}	2.12 ^{ns}	6.43 ^{**}	7.64 ^{**}

^{1/1} in each column, mean ($N = 4$) followed by the different lower case letter in the same column are significantly different ($p \leq 0.05$).

^{**}Significant difference ($p \leq 0.01$).

^{ns}Not significant difference.

CV = coefficient of variation.

LSD_{0.05} = Least Significant Difference at $\alpha = 0.05$.

LSD_{0.01} = Least Significant Difference at $\alpha = 0.01$.

Daft, 1990; Bethlenfalvai and Linderman, 1992; Medeiros et al., 1994; Osnubi, 1994; Ortas et al., 1996).

The highest biomass (57.2 g/plant) and percentage of nitrogen uptake in sorghum shoots (1.0%) resulted from the treatment with *A. scrobiculata*. Barea et al. (1987) and Azco'n Aguilar et al. (1993) reported a high nitrogen uptake in plants inoculated with AM fungi. The total absorption surface of infected plant roots has been reported to be effectively increased by AM fungal mycelia and this improved the plant's access to nutrients (Lambert et al., 1979; Ortas et al., 1996; George, 2000; Cavagnaro et al., 2015). Furthermore, more recent studies have shown that AM fungi have other agriculturally valuable functions—reducing P and N loss from the soil by a reduction in the leaching of PO_4^- and NH_4^+ or NO_3^- or both (van der Heijden, 2010; Asghari and Cavagnaro, 2011). Douds and Reider (2003) found that inoculation with AM fungi increased the

Table 4
Percentage and the uptake of phosphorus in *Sorghum bicolor* Linn. (KU 439).

Treatment	% Phosphorus		Phosphorus uptake (mg/plant)	
	Shoots	Roots	Shoots	Roots
Control	0.26 ^{d/1}	0.15 ^{cd/1}	6.50 ^{d/1}	0.87 ^{d/1}
<i>Glomus</i> sp. 1	0.44 ^a	0.18 ^{abc}	13.95 ^a	1.03 ^{cd}
<i>Glomus</i> sp. 2	0.26 ^d	0.14 ^d	7.25 ^d	1.07 ^{bcd}
<i>Glomus</i> sp. 3	0.31 ^{bcd}	0.16 ^{cd}	9.02 ^{bcd}	0.96 ^{cd}
<i>G. aggregatum</i>	0.33 ^{bcd}	0.16 ^{cd}	10.52 ^{bc}	1.18 ^{bcd}
<i>G. fasciculatum</i>	0.28 ^{cd}	0.20 ^a	8.51 ^{cd}	1.26 ^{abc}
<i>A. longula</i>	0.26 ^d	0.19 ^{ab}	7.65 ^d	1.50 ^a
<i>G. occultum</i>	0.26 ^d	0.16 ^{cd}	7.85 ^d	0.98 ^{cd}
<i>A. scrobiculata</i>	0.39 ^{ab}	0.18 ^{abc}	12.96 ^{ab}	1.39 ^{ab}
<i>A. spinosa</i>	0.37 ^{abc}	0.17 ^{bc}	14.50 ^a	1.12 ^{bcd}
<i>Scutellospora</i> sp.	0.36 ^{abc}	0.16 ^{cd}	12.40 ^{ab}	1.10 ^{bcd}
CV	17.7	10.00	27.30	18.00
LSD _{0.05}	0.08	0.24	3.97	0.29
LSD _{0.01}	0.10	0.03	5.33	0.39
F-test	4.94 [*]	4.13 ^{**}	4.36 ^{**}	3.48 ^{**}

Notes: ^{1/1} in each column, mean ($N = 4$) followed by the different lower case letter in the same column are significantly different ($p \leq 0.05$).

^{*}Significant difference ($p \leq 0.05$).

^{**}Significant difference ($p \leq 0.01$).

CV = coefficient of variation.

LSD_{0.05} = Least Significant Difference at $\alpha = 0.05$.

LSD_{0.01} = Least Significant Difference at $\alpha = 0.01$.

percentage of nitrogen uptake and yield of green pepper even when grown in a soil with high available P.

The results of the growth of sorghum in soil inoculated with 10 different species of native AM fungi showed that *A. scrobiculata* inoculation produced a high percentage of sorghum root colonization similar to *A. spinosa* and *Glomus* sp. 1 inoculation. *A. spinosa* produced the highest percentage of root colonization and phosphorus uptake in shoots. *Scutellospora* sp. gave the highest shoot dry weight (34.34 g/plant) and potassium uptake (45.34 mg/plant) in sorghum shoots. Nevertheless, the greatest number of spores in the soil (14.10 spore/g soil), number of leaves (10.30 leaves/plant), grain dry weight (16.05 g/plant), biomass (57.24 g/plant), percentage of nitrogen in shoots (1.01% N) and total nitrogen uptake in shoots (33.65 mg/plant) were found in *A. scrobiculata* inoculation. Therefore, the authors recommend that future experiments should be conducted in the field using *A. scrobiculata* inoculation to

Table 5
Percentage and the uptake of potassium in *Sorghum bicolor* Linn. (KU 439).

Treatment	% Potassium		Potassium uptake (mg/plant)	
	Shoots	Roots	Shoots	Roots
Control	1.13 ^{cd/1}	1.19	28.34 ^{d/1}	6.53 ^{c/1}
<i>Glomus</i> sp. 1	1.34 ^{abc}	1.07	42.83 ^{abc}	6.88 ^c
<i>Glomus</i> sp. 2	1.37 ^{ab}	1.28	38.23 ^{c-d}	10.30 ^a
<i>Glomus</i> sp. 3	1.15 ^{bcd}	1.26	33.08 ^{cd}	7.34 ^{bc}
<i>G. aggregatum</i>	1.27 ^{a-d}	1.19	40.72 ^{abc}	8.80 ^{abc}
<i>G. fasciculatum</i>	1.12 ^{cd}	1.20	41.27 ^{abc}	7.50 ^{bc}
<i>A. longula</i>	1.38 ^a	1.20	41.40 ^{abc}	9.50 ^{ab}
<i>G. occultum</i>	1.11 ^d	1.21	33.91 ^{bcd}	7.32 ^{bc}
<i>A. scrobiculata</i>	1.34 ^{abc}	1.02	44.74 ^{ab}	7.97 ^{abc}
<i>A. spinosa</i>	1.33 ^{abc}	1.11	43.27 ^{abc}	7.43 ^{bc}
<i>Scutellospora</i> sp.	1.27 ^{a-d}	1.25	45.34 ^a	8.71 ^{abc}
CV	10.80	11.80	17.30	19.40
LSD _{0.05}	0.19	0.20	9.82	2.24
LSD _{0.01}	0.26	0.26	13.19	3.00
F-test	2.47 ^{**}	1.39 ^{ns}	2.50 ^{**}	2.24 ^{**}

^{1/1} in each column, means ($N = 4$) followed by the different lower case letter in the same column are significantly different ($p \leq 0.05$).

**Significant difference ($p \leq 0.01$).

^{ns}Not significant difference.

CV = coefficient of variation.

LSD_{0.05} = Least Significant Difference at $\alpha = 0.05$.

LSD_{0.01} = Least Significant Difference at $\alpha = 0.01$.

enhance sorghum growth, nutrient uptake and grain production. The use of this AM fungi as a biofertilizer is an alternative to enhance organic agriculture and would introduce environmentally friendly farming practices.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was financially supported by the Kasetsart University Research and Development Institute (KURDI) (grant no. 04111387), Bang Kean, Bangkok, Thailand. The authors would like to express their sincere thanks to Assoc. Prof. Poonpilai Suwanarit from the Department of Microbiology, Prof. Dr. Ngampong Kongkathip from the Department of Chemistry, Faculty of Science, Kasetsart University, Thailand and Ms. Yadana Nath Desmond, CPRE, Teachers College Columbia University, USA, for fruitful discussion and valuable comments.

References

- Allen, M.F., Sexton, J.C., Moore, T.S., Christensen, M., 1981. Influence of phosphate source on vesicular arbuscular mycorrhizae of *Bouteloua gracilis*. *New Phytol.* 87, 687–691.
- Asghari, H.R., Cavagnaro, T.R., 2011. Arbuscular mycorrhizas enhance plant interception of leached nutrients. *Funct. Plant Biol.* 38, 219–226.
- Augé, R.M., 2001. Water relations, drought and VA mycorrhizal symbiosis. *Mycorrhiza* 11, 3–42.
- Azco'n Aguilar, C., Alba, C., Montilla, M., Barea, J.M., 1993. Isotopic (¹⁵N) evidence of the use of less available N forms by VA mycorrhizae. *Symbiosis* 15, 39–48.
- Bagayoko, M., George, E., Römhelt, V., Buerkert, A., 2000. Effects of mycorrhizae and phosphorus on growth and nutrient uptake of millet, cowpea and sorghum on a West African soil. *J. Agr. Sci.* 135, 399–407.
- Barea, J.M., Azco'n Aguilar, C., Azco'n, R., 1987. Vesicular-arbuscular mycorrhiza improve both symbiotic N₂ fixation and N uptake from soil as assessed with a ¹⁵N technique under field conditions. *New Phytol.* 106, 717–725.
- Beever, R.E., Burns, D.J.W., 1980. Phosphorus uptake, storage and utilization by fungi. *Adv. Bot. Res.* 8, 127–219.
- Bethlenfalvy, G.J., Linderman, R.G., 1992. Mycorrhizae in Sustainable Agriculture. American Society of Agronomy, Inc., America, p. 124.

- Boyetchko, S.M., Tewari, J.P., 1990. Root colonization of different hosts by the vesicular arbuscular mycorrhizal fungus *Glomus dimorphicum*. *Plant Soil* 129, 131–136.
- Cavagnaro, T.R., 2008. The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations. *Plant Soil* 304, 315–325.
- Cavagnaro, T.R., Franz Bender, S., Asghari, H.R., van der Heijden, M.G., 2015. The role of arbuscular mycorrhizas in reducing soil nutrient loss. *Trends Plant Sci.* 20, 283–290.
- Chapman, H.D., Pratt, P.F., 1978. *Methods of Analysis for Soils Plants and Waters*. Division of Agricultural Science, University of California, Los Angeles, CA, USA, p. 309.
- Cress, W.A., Throneberry, E.D., Lindsey, D.L., 1979. Kinetics of phosphorus absorption by mycorrhizal and non mycorrhizal tomato root. *Plant Physiol.* 64, 484–487.
- Dodd, J.C., Burton, C.C., Burns, R.G., Jeffries, P., 1987. Phosphatase activity associated with the roots and the rhizosphere of plants infected with vesicular arbuscular mycorrhizal fungus. *New Phytol.* 107, 163–172.
- Douds Jr., D.D., Reider, C., 2003. Inoculation with mycorrhizal fungi increases the yield of green peppers in a high P soil. *Biol. Agric. Hortic.* 21, 91–102.
- Farzaneh, M., Vierheilig, H., Lössl, A., Kaul, H.P., 2011. Arbuscular mycorrhiza enhances nutrient uptake in chickpea. *Plant Soil Environ.* 57, 465–470.
- George, E., 2000. Contribution of arbuscular mycorrhizal fungi to plant mineral nutrition. In: Kapulnik, Y., Douds Jr., D.D. (Eds.), *Arbuscular Mycorrhizas: Physiology and Function*. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 107–129.
- George, E., Römhelt, V., Marschner, H., 1994. Contribution of mycorrhizal fungi to micronutrient uptake by plants. In: Manthey, J.A., Crowley, D.E., Luster, D.G. (Eds.), *Biochemistry of Metal Micronutrients in the Rhizosphere*. CRC Press, Boca Raton, Florida, pp. 93–109.
- Gerdemann, J.W., Nicolson, T.H., 1963. Spore of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* 46, 235–244.
- Guo, W., Zhao, R., Zhao, W., Fu, R., Guo, J., Bi, N., Zhang, J., 2013. Effect of arbuscular mycorrhizal fungi on maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) grown in rare earth elements of mine tailings. *Appl. Soil Ecol.* 72, 85–92.
- Hadley, H.H., 1957. An analysis of variation in height in sorghum. *Agron. J.* 49, 144–147.
- Harrison, M.J., 1997. The arbuscular mycorrhizal symbiosis: an underground association. *Trends Plant Sci.* 2, 54–60.
- ICRISAT, 2009. Sorghum. International Crops Research Institute for the Semi-Arid Tropics, Andhra Pradesh, India. Available from: <http://www.icrisat.org/sorghum/sorghum.htm> (accessed 29.04.09).
- Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. In: Schenck, N.C. (Ed.), *Methods and Principles of Mycorrhizal Research*, third ed. APS Press, St Paul, MN, USA.
- Kothari, S.K., Marschner, H., Römhelt, V., 1990. Direct and indirect effects of VA mycorrhizae and rhizosphere microorganisms on mineral nutrient acquisition by maize (*Zea mays* L.) in a calcareous soil. *New Phytol.* 116, 637–645.
- Lambert, D.H., Baker, D.E., Cole Jr., H., 1979. The role of mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. *Soil Sci. Soc. Am. J.* 43, 976–980.
- Lehmann, A., Stavros, D.V., Leifheit, E.F., Rillig, M.C., 2014. Arbuscular mycorrhizal influence on zinc nutrition in crop plants: a meta-analysis. *Soil Biol. Biochem.* 60, 123–131.
- Lendzemo, V.W., Kuyper, T.W., 2001. Effects of arbuscular mycorrhizal fungi on damage by *Striga hermonthica* on two contrasting cultivars of sorghum, *Sorghum bicolor*. *Agric. Ecosyst. Environ.* 87, 29–35.
- Marschner, H., Dell, B., 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* 159, 89–102.
- McArthur, D.A.J., Knowles, N.R., 1993. Influence of VAM and phosphorus nutrition of potato. *Plant Physiol.* 102, 771–782.
- Medeiros, C.A.B., Clark, R.B., Ellis, J.R., 1994. Growth and nutrient uptake of sorghum cultivated with vesicular-arbuscular mycorrhiza isolates at varying pH. *Mycorrhiza* 4, 185–191.
- Miller, R.M., Jastrow, J.D., 2000. Mycorrhizal fungi influence soil structure. In: Kapulnik, Y., Douds, D.D. (Eds.), *Arbuscular Mycorrhizas: Physiology and Function*. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 3–18.
- Ortas, I., Harris, P.J., Powell, D.L., 1996. Enhanced uptake of phosphorus by mycorrhizal sorghum plants influenced by formed of nitrogen. *Plant Soil* 184, 2255–2264.
- Osnubi, O., 1994. Comparative effects of vesicular-arbuscular mycorrhizal inoculation and phosphorus fertilization on growth and uptake of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) plants under drought-stress conditions. *Biol. Fertil. Soils* 18, 55–59.
- Pearson, V., Tinker, P.B., 1975. Measurement of phosphorus fluxes in the external hyphae of endomycorrhizae. In: Sanders, F.E., Mosse, B., Tinker, P.B. (Eds.), *Endomycorrhizas*. Academic Press, New York, NY, USA.
- Raju, P.S., Clark, R.B., Ellis, J.R., Maranville, J.W., 1990. Mineral uptake and growth of sorghum colonized with VA mycorrhiza at varied soil phosphorus levels. *J. Plant Nutr.* 13, 843–859.
- Schenck, N.C., Pérez, Y., 1988. *Manual for the Identification of VA Mycorrhizal Fungi*, second ed. INVAM, University of Florida, Gainesville, FL, USA.
- Simpson, D., Daft, M.J., 1990. Spore production and mycorrhizal development in various tropical crop hosts infected with *Glomus clarum*. *Plant Soil* 121, 171–178.

- Smith, S.E., Read, D.J., 1997. *Mycorrhizal Symbiosis*, second ed. Academic Press, London, UK, p. 605.
- Subramanian, K.S., Charet, C., Dwyer, L.M., Hamilton, R.I., 1995. Arbuscular mycorrhizas and water relations in maize under drought stress at fasselling. *New Phytol.* 129, 643–650.
- Techapinyawat, S., Suwannarit, P., Pakkong, P., Sinbuathong, N., Sumthong, P., 2000. Selection of effective vesicular-arbuscular mycorrhizal fungi on growth and nutrient uptake of vetiver. *Thai J. Agric. Sci.* 34, 91–99.
- Tewari, L., Johri, B.N., Tandom, S.M., 1993. Host genotype dependency and growth enhancing ability of VA-mycorrhizal fungi for *Eleusine coracana* (finger millet). *World J. Microbiol. Biotechnol.* 9, 191–195.
- Tobar, R., Azcon, R., Barea, J.M., 1994. Improved nitrogen uptake and transport from ¹⁵N-labelled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. *New Phytol.* 126, 119–122.
- Trouvelot, A., Kough, J.L., Gianinazzi-Pearson, V., 1986. Mesure du taux de mycorrhization ayant une signification fonctionnelle. In: Gianinazzi-Pearson, V., Gianinazzi, S. (Eds.), *Physiological and Genetic Aspects of Mycorrhizae*. INRA, Paris, France.
- van der Heijden, M.G., 2010. Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. *Ecology* 91, 1163–1171.
- Vázquez, M.M., César, S., Azcón, R., Barea, J.M., 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. *Appl. Soil Ecol.* 15, 261–272.