



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

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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*.

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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

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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 occulatum*, *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: Chlamydospores 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: Chlamydospores 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: Chlamydospores 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, 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 fibrous 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

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