Applied Soil Ecology 119 (2017) 384–391

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Combined effects of arbuscular mycorrhiza and drought stress on plant growth and mortality of forage sorghum

Xueguang Sun[a,c,*], Jing Shi[b], Guijie Ding[a,c]

[a] Institute for Forest Resources & Environment of Guizhou, Guizhou University, Guiyang, 550025, China
[b] Guizhou Minzu University, Guiyang, 550025, China
[c] College of Forestry, Guizhou University, Guiyang, 550025, China

ARTICLE INFO

Keywords:
Abuscular mycorrhiza
Forage sorghum
Progressive drought stress
Biomass
Mortality

ABSTRACT

Drought stress affects the growth and mortality of forage sorghum cultivated in arid and semi-arid environments. Arbuscular mycorrhizal (AM) fungi can promote plant growth, protect host plants from various stresses, and mediate plant–plant interactions. In this study, a greenhouse experiment was conducted to investigate: (1) the function of an AM fungus (*Funneliformis mosseae*) in the alleviation of drought-induced growth retardation and mortality of forage sorghum plants, and (2) whether common mycorrhizal networks (CMNs) are more beneficial to plants that join CMNs earlier than to those that join later. AM formation largely promoted sorghum growth, and the biomass and the specific leaf area (SLA) of mycorrhizal plants were larger than those of non-mycorrhizal plants, regardless of whether they were grown under well-watered or drought stress conditions. Progressive drought stress suppressed plant growth and even induced plant mortality, while AM alleviated plant growth retardation and prolonged plant lifespan. Intriguingly, plants connected to the CMN and that formed an AM symbiosis earlier than other plants benefited more under intensive drought stress, with longer lifespans, more arbuscules and larger ratios of intact arbuscules. These findings may help to elucidate the seemingly random death of individual plants induced by drought stress in agricultural practices. The plants that formed AM earlier than other plants were not significantly superior in terms of biomass accumulation, SLA and AM colonization under well-watered conditions. Our results confirmed that the formation of an AM association could alleviate growth retardation and prolong the lifespan of plants under drought stress, demonstrating the prospect of utilizing AM fungi to increase the forage yield of sorghum plants cultivated under semi-arid and arid environments.

1. Introduction

The cultivation of a single crop species as a plantation is common practice in modern agroecosystems, which often leaves the crop susceptible to a variety of biotic and abiotic stresses (Weston et al., 2013). Water depletion is among the most frequent abiotic stresses that limit crop production (including sorghum) in arid and semi-arid areas (Zhang et al., 2006). Severely limited water availability can induce developmental delays, reduce plant dry weight and even induce mortality (Rees, 1986). Drought-induced plant death has frequently been observed; however, intriguingly, some plants are able to survive while others succumb to drought (McDowell et al., 2008), which is analogous to random events.

As a result of water limitation, plants can express various responses and have developed a wide diversity of morphological and physiological drought tolerance mechanisms (Blum, 1996; Ruiz-Lozano et al., 2016). Furthermore, many plants can form an arbuscular mycorrhizal (AM) symbiosis with fungi of the Glomeromycota and have been widely reported to improve the hosts' tolerance to various stresses (e.g., Abbaspour et al., 2012). An AM is a mutualistic association between an AM fungus and the roots of a plant, in which the fungal partner facilitates the acquisition of mineral nutrients by the plant and the host provides carbohydrates to the fungus (Smith and Read, 2008). Studies on a range of different plants have shown that the formation of an AM can improve the host's drought tolerance through alleviating H_2O_2 and malondialdehyde accumulation (Benhiba et al., 2015), enhancing antioxidant enzyme activities (Benhiba et al., 2015) and the soluble sugar accumulation of host plants (Yooyongwech et al., 2013), regulating the expression of aquaporin genes of both symbionts (Bárzana et al., 2014; Li et al., 2013), improving host P uptake (Li et al., 2015), leaf photosynthesis (Gehring et al., 2017; Li et al., 2015) and plant biomass (Ruiz-Lozano et al., 2016), as well as altering the hydraulic properties of plant roots (Zhao et al., 2015). Most of the previous research regarding the role of AM in improving the host's tolerance to drought stress have

* Corresponding author at: Institute for Forest Resources & Environment of Guizhou, Guizhou University, Guiyang, 550025, China.
E-mail address: sunxg0518@aliyun.com (X. Sun).

http://dx.doi.org/10.1016/j.apsoil.2017.07.030
Received 5 April 2017; Received in revised form 16 July 2017; Accepted 18 July 2017
Available online 28 July 2017
0929-1393/

focused on the host's status, whereas the status of the AM under drought stress, particularly the status of the arbuscules, has received little attention. The arbuscule is the key structure in the AM symbiosis where mineral nutrients and carbohydrates as well as water are exchanged between the symbionts (Garcia et al., 2016; Li et al., 2013). The amount of arbuscules and their intactness may directly reflect the function of the AM.

AM fungal mycelium can form extensive networks in the soil and form connections with multiple host plants; these common mycorrhizal networks (CMNs) could affect the survival and fitness of individual host plants under stress (Bücking et al., 2016). Extraradical mycelia of mycorrhizal fungi could uptake and transport water directly to host plants from the soil and even from space far from the root tips to alleviate plant water deficit (Wu et al., 2013). The higher relative water content in the colonized roots and increased expression of the functional aquaporin genes in extraradical mycelia under drought stress further highlighted the fundamental role of CMNs in plant drought tolerance (Li et al., 2013). Weremijewicz et al. (2016) claimed that CMNs preferentially allocate more mineral nutrients and perhaps also more water to larger host plants than to smaller host plants of the same species. Besides this size effect, we hypothesize that plants of the same size but with different levels of mycorrhization (i.e., different amounts of functional arbuscules) owing to the length of time that they have been connected to the CMN, may also perform differently under drought stress. And it is common that plants of similar sizes generally form AM at distinct times in agricultural practices.

As one of the most important fresh fodder and silage sources in the world, *Sorghum bicolor* (L.) Moench is extensively grown as a forage crop and is becoming increasingly important in many regions of the world (Cothren et al., 2000; Miron et al., 2006). Many cultivars of forage sorghum have been cultivated, among which, the cultivar Hunnigreen is widely planted in western China due to its high nutritive value and productivity, long growth period and late and seldom/never flowering properties (Qu et al., 2014). The effects of AM formation on the performance of grain sorghum are well known (Cho et al., 2006; Raju et al., 1990). By contrast, little is known about the regulation of plant fitness and mortality of forage sorghum by AM fungi under intensive drought stress. Furthermore, very few studies have measured both fungal and plant performance during drought conditions (Gehring et al., 2017). In this paper, a greenhouse experiment was conducted to investigate: (1) the function of AM in the alleviation of drought-induced growth retardation and mortality of sorghum plants; and (2) whether CMNs are more beneficial to plants that form mycorrhiza earlier than to those that form mycorrhiza later. Sorghum plants were inoculated with mono AM fungal inoculums under different mycorrhization regimes. After AM establishment, plants were subjected to drought stress or continued to receive an adequate water supply. Plant biomass accumulation and partition, specific leaf area (SLA) and plant mortality and AM status were recorded to investigate the potential functions of the AM and/or the CMN in terms of regulating the productivity and mortality of forage sorghum under progressive drought stress.

2. Materials and methods

2.1. AM fungus and plant materials

The AM fungal inoculant *Funneliformis mosseae* (BGC NM01A), which was bought from the Bank of Glomales in China, was used as the inoculum. The inoculum comprised spores, mycorrhizal fragments and infested soil. For mycorrhizal treatments (M and MD treatments, Fig. 1), five tablespoons of inoculant were added to the soil surrounding plant root in each microcosm compartment (approximately 5 g of inoculant per plant). Each gram of *F. mosseae* inoculant contained 40 spores. The number of spores per gram of soil was determined using a wet sieving and decanting method (Gerdemann and Nicolson, 1963). Fornon-mycorrhizal control (NM treatment, Fig. 1), plants were given the same amount of autoclaved mycorrhizal inoculum together with a 5 mL aliquot of a filtrate (< 20 μm) of the AM inoculum in order to provide a general microbial population free of AM propagules.

Seeds of forage sorghum, which were bought from a local supplier (cultivar Hunnigreen, Barenbrug Beijing International Grass Co., Ltd.), were surface sterilized with 5% H_2O_2-solution for 5 min and then rinsed with sterilized distilled water. The seeds were then left to germinate and grow in sterilized river sand supplied with tap water only. After the seedlings had developed two leaves each, two uniform seedlings were transplanted to each of the microcosms (Fig. 1) containing the sterilized soil and the live or sterile AM fungal inoculum.

2.2. Growth substrates

The sorghum plantlets were planted in Loessial sandy soil collected from Baijie County, Shaanxi Province (a typical semi-arid region in China with an annual precipitation of less than 400 mm). The soil was first passed through a 2 mm sieve and then autoclaved under pressure (0.11 MPa) at 121 °C for 2 h. The basic physical and chemical properties of the soil (after sterilization) were as follows: pH 7.7, 7.9 g kg^{-1} of soil organic matter, 0.97 g kg^{-1} of total nitrogen, 25.7 mg kg^{-1} of available nitrogen, 0.68 g kg^{-1} of total phosphorus, 3.05 mg kg^{-1} of available phosphorus, and 62.7 mg kg^{-1} of available potassium. In total, 2.5 L of soil was placed in each microcosm compartment; to minimize evaporation, the soil surface was covered with polythene.

2.3. Experimental design and plant growth conditions

The experiment was a 4 × 2 complete factorial combination comprising four mycorrhization strategies (Fig. 1) and two watering regimes (drought and well-watered treatments). The experiment was laid out in a randomized complete block design. In total, 125 dual-compartment microcosms were used: 35 microcosms for plants inoculated with sterile inoculum (NM treatment), 35 for plants inoculated with the AM fungus (M treatment), and 55 for the AM receiver plants (MT) and the AM donor plants (MD) (one plant was grown in each compartment, i.e., in total, 250 sorghum plants were used).

Plants were grown in a glasshouse under natural day/night conditions. Each microcosm was watered to 80% of field capacity every other day (each compartment received the same amount of water). 50 mL of Hoagland solution (containing only half the concentration of phosphorus normally present in Hoagland solution, i.e., 0.5 mmol L^{-1} PO_4^{-3}) was added to each compartment; this procedure was repeated at the beginning of each week for the first four weeks. This low concentration of P improves plant growth while maintaining high levels of arbuscular mycorrhizal colonization (Gao, 2002). Microcosms were rearranged weekly to provide random distribution in the greenhouse (to avoid positional effects).

At the end of the fourth week, the septum between the two compartments of each microcosm was removed and the nutritional supplements were stopped. At the end of the 10th week, each of the four mycorrhization treatments was assessed: five of the microcosms in each treatment were randomly selected and the sorghum plants were measured to determine their biomass, SLA and the formation of AM.

Ten weeks post transplantation, the remanent microcosms in each mycorrhization treatment were equally divided into two subgroups and were either subjected to drought stress (water supplements were stopped to simulate progressive drought stress in a natural arid environment) or received the normal water supplement (80% of field capacity). The status of the AM and plant growth were assessed on the 10th day (i.e. 80 days post transplatation) after the start of the drought stress treatment (five of the microcosms in each treatment were randomly selected); plant mortality was checked every day in the drought stress subgroup until all the plants were dead (n = 20). The mean temperature during the growth period was 28 °C.