

Impact of arbuscular mycorrhizal fungi on the growth, water status, and photosynthesis of hybrid poplar under drought stress and recovery

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Abstract

Poplars (*Populus* spp.) are widely used in the pulp and paper industry and as bioenergy resources. Poplars require a large amount of water for biomass accumulation and lack of water is a limiting factor for poplar growth. Arbuscular mycorrhizal (AM) fungi have been previously reported to afford some plant species with greater resistance to drought stress. However, the effects of AM fungi on hybrid poplar under drought stress and recovery have not been studied. The main aim of this study was to evaluate the effects of the AM fungus, *Rhizophagus irregularis*, on the growth, water status, chlorophyll (Chl) content and fluorescence, and photosynthesis of poplar seedlings. The experiment was divided into three stages. At each stage of the experiment, the seedlings were subjected to a different watering regime: well-watered (prior stress), drought, and then rewatering (recovery). Measurements were taken at the end of each stage of the experiment. The results showed that mycorrhizal plants had a higher net photosynthetic rate and Chl fluorescence compared with nonmycorrhizal plants, regardless of the stage. Mycorrhizal and nonmycorrhizal plants showed different responses to drought stress: mycorrhizal plants showed better water-use efficiency and water uptake under drought stress conditions. In general, the poplar seedlings that formed the AM symbiosis with *R. irregularis* showed enhanced growth and reduced loss of biomass during the drought stress compared with the nonmycorrhizal seedlings.

Additional key words: drought tolerance; gas exchange; nonphotochemical quenching; photosynthetic capacity; relative water content.

Introduction

Drought, one of the most frequent and severe abiotic stress factors, limits plant growth and crop productivity in many arid and semiarid regions (Qiao *et al.* 2012, Zhu *et al.* 2012). Dryland ecosystems cover over 35% of the world terrestrial land mass (Housman *et al.* 2006). Furthermore, seasonal droughts often occur even in the nonarid regions (Zhu *et al.* 2012). Therefore, it is crucial to understand the mechanisms that plants use to respond to drought and how they recover from drought stress conditions (Vaňková *et al.* 2012).

Arbuscular mycorrhizal (AM) fungi are able to form a symbiotic relationship with 80% of terrestrial plant species – an association that is mutually beneficial to the host plant and the fungus (Smith and Read 2008). Many studies have observed that under drought stress conditions, plants that have formed a symbiotic relationship with AM fungi have enhanced growth and are more resistant to drought (Augé 2001, Wu and Xia 2006). This is because mycorrhizal plants have increased chlorophyll (Chl) content and photosynthetic ability (Shrestha *et al.* 1995,

Received 14 December 2013, accepted 18 August 2014.

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Abbreviations: AM – arbuscular mycorrhizal; Chl – chlorophyll; C_i – intercellular CO₂ concentration; DS – drought stress; E – transpiration rate; F_0 – the minimal fluorescence in dark-adapted state; F_0' – the minimal fluorescence in light-adapted state; F_m – the maximal fluorescence in dark-adapted state; F_m' – the maximal fluorescence in light-adapted state; F_s – the steady-state fluorescence; F_v/F_m – the maximal quantum yield of PSII in dark-adapted state; g_s – stomatal conductance; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; REC – recovery; RWC – relative water content; WUE – water-use efficiency; WW – well-watered; Φ_{PSII} – effective quantum yield of PSII.

Acknowledgments: This research was supported by Special Fund for Forest Scientific Research in the Public Welfare (201404217), the National Natural Science Foundation of China (31270639 and 31170567), the Program for Changjiang Scholars and Innovative Research Team in University of China (IRT1035), and the Ph.D. Program Foundation of the Education Ministry of China (20110204130001). We also thank the anonymous reviewers for reviewing the manuscript and offering helpful suggestions.

Wu and Xia 2006, Sheng *et al.* 2009, Zai *et al.* 2012, Gong *et al.* 2013). AM fungi are able to promote the water and physiological status of the host by altering the rate of water movement in the host plant (Augé 2001, Sikes *et al.* 2010), and by affecting tissue hydration (Aroca *et al.* 2008).

Poplars (*Populus* spp.) are diverse and widely distributed forest trees (Regier *et al.* 2009) that are important commercially (Xiao *et al.* 2009) because they can be used as bioenergy resources (Luo and Polle 2009) and as materials in the pulp and paper industry (Ai and Tschirner 2010). High water availability is important for poplar biomass production (Cao *et al.* 2012). However, poplars belong to one of the most drought-sensitive woody plant groups (Xiao *et al.* 2009); hence, water shortage is a key limiting factor for poplar growth.

AM fungi have been widely used as a biofertilizer to increase the plant yield, and to help crops withstand the adverse environmental conditions that occur in agriculture and forestry programmes (Johansson *et al.* 2004, Jiang *et al.* 2013). Several studies have revealed that AM fungi can form a symbiosis with a range of *Populus* species (Khasa *et al.* 2002, Quoreshi and Khasa 2008, Rooney *et al.* 2011), resulting in increased *Populus* growth, nutrient uptake and resistance, particularly under adverse

Materials and methods

Growing conditions and plant material: A mixture of dry soil and fine sand (1:2, v/v) was used as a growth substrate. The soil used in this study was collected from the top layer (0–20 cm) of a field in Yangling City, Shaanxi Province, China. Soil pH was 7.5 (measured at a soil:water ratio of 1:2.5). The soil contained 35.8 mg(N) kg⁻¹, 11.3 mg(P) kg⁻¹, 158.6 mg(K) kg⁻¹, and 18.6 g kg⁻¹ of organic matter. The soil was air dried, sieved through a 2 mm sieve, and subsequently mixed with fine sand. The soil and sand mixture was sterilized by autoclaving at 121°C for 2 h. Cuttings (15 cm) of *Populus × canadensis* (a hybrid of *P. nigra × P. deltoides*) ‘Neva’ were collected from a nursery in Yangling City, Shaanxi Province, China, surface disinfected for 15 s in 70% (v/v) ethanol, rinsed in sterile deionized water five times, and planted immediately in pots (22.5 cm × 22.5 cm) containing 4 kg of the substrate.

AM inoculum: We carried out a preliminary experiment and selected the more efficient AM fungus strain *Rhizophagus irregularis* from three widely used AM fungus (*R. irregularis*, *Glomus versiforme*, and *G. mosseae*). *R. irregularis* is the AM fungus strain, where the genome has been completely sequenced. Hence, the AM fungus used as the inoculum was *R. irregularis* (Błaszczak, Wubet, Renker & Buscot) Walker & Schüßler (BGC BJ09), provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, China. The inoculum consisted of spores (50 spores per g of dry inoculant), mycelium, root fragments, and soil.

environmental conditions (Cicatelli *et al.* 2010). Poplar can be colonized by both ectomycorrhizal (ECM) and AM fungi in nature (Lopez-Aguillon and Garbaye 1990). Previous studies have investigated whether ECM fungi enable poplar to resist drought stress; the results showed that ECM fungi could help poplar to avoid stress via increasing the radial stem and vessel cross sectional areas (Beniwal *et al.* 2010) and improving the water transport capacity of poplar (Marjanović *et al.* 2005). AM colonization is dominant in young poplar seedlings (Lopez-Aguillon and Garbaye 1990, Quoreshi and Khasa 2008). The health of young seedlings is critical for the subsequent seedling growth. However, the effect of AM fungi on hybrid poplar has not been examined under drought stress and recovery. Hence, the main objective of this study was to evaluate the effect of inoculating hybrid poplar seedlings with the AM fungus *R. irregularis* on the growth, water status, Chl content, Chl fluorescence, and gas exchange under drought stress and when rewatered (recovery from drought stress). We also investigated whether hybrid poplar seedlings increased their drought tolerance when grown in pots under greenhouse conditions and inoculated with *R. irregularis*.

Experimental design: The experiment was conducted in a greenhouse with 12-h light period at a temperature of 25–35°C. Pots were arranged in a randomized complete block design. Treatments included two factors: (1) the inoculation with *R. irregularis* or the nonmycorrhizal control, and (2) the water status, either well-watered (WW) throughout the experiment or drought period (DS) during the course of the experiment. In order to prevent the individual differences and the unexpected death of the seedlings, which would influence the experiment, we prepared 30 replicates per treatment. At the 90th day of the experiment, 30 mycorrhizal poplars and 30 non-mycorrhizal poplars were randomly selected, for the next experiment.

The experiment was carried out in three stages: the plants were well-watered (WW) for the first 90 d prior stress (PS, during this period the AM colonization rate reached a stable point), then they were subjected to drought stress (DS) for the next seven days (seedlings began wilting), and then rewatered (REC) for the next 30 d (seedlings overcame the stress and resumed growth). During the WW treatment, the soil water content was kept at 75% of field capacity; during the DS treatment, the soil water content was kept at 50% of field capacity by weighing the pots every day to control soil water content. A preliminary experiment was carried out to fine-tune the DS period (data not shown). After 7 d period of drought at 50% field capacity, the poplar seedlings began wilting; defoliation appeared when they received severer drought (40% of field capacity). Hence, we chose the 50% of the

field capacity and DS for 7 d in order to allow plants to fully recover after this stress period.

Plants treated with the AM were inoculated with 5 g of inoculum per pot and the control plants were inoculated with 5 g of the autoclaved inoculum together with 10 mL of inoculum washing solution filtered to remove AM propagules. At the end of each of the three soil-watering stages, six plants were randomly selected to determine stem length, relative Chl content, Chl fluorescence parameters, and gas-exchange parameters, and then harvested to determine root colonization, dry mass, and relative water content (RWC).

Frequency of AM colonization: Roots from each treatment were collected and washed gently under running tap water and then rinsed with distilled water. A subsample of 0.5 g was taken from each root and cut into 1 cm long segments, bleached for 30 min in 10% KOH at 90°C, acidified in 1% HCl for 10 min, and dyed with Trypan blue (Phillips and Hayman 1970). Two hundred root segments per treatment were examined to determine the proportion of roots that had been colonized by the AM fungus.

Growth characteristics and RWC measurements: Stem length was measured with a ruler. After harvest, leaves, shoots, and roots were first placed in an oven at 105°C for 20 min to destroy the enzymes and then dried at 80°C to constant mass to determine the biomass of the leaves, shoots, and roots.

The RWC of poplar leaves was measured following the method used by Castillo (1996) and calculated as: $RWC [\%] = (FM - DM)/(TM - DM) \times 100$, where FM is fresh mass, TM is turgid mass (mass after leaf samples were soaked in distilled water for 24 h), and DM is dry mass.

Relative Chl content and Chl fluorescence parameters: The fifth fully expanded leaf (from the apex) was used to measure the relative Chl content using a SPAD meter

Results

Frequency of AM colonization and plant growth: None of the poplar seedlings that received the nonmycorrhizal treatment was colonized by *R. irregularis* during the whole experiment (Table 1). During the experiment, the frequency of AM colonization of poplar seedlings was 85.6–87.1% under WW condition, and 85.6–87.9% under DS condition. The frequency of AM colonization did not change during the PS, DS, and REC stages under both water conditions (Table 1).

By the end of the PS stage (day 90), poplar seedlings colonized by *R. irregularis* exhibited significantly greater stem length (113.8%), leaf dry mass (35.7%), stem dry mass (51.9%), and root dry mass (29.6%), compared with the nonmycorrhizal seedlings (Tables 2, 3).

By the end of the DS stage (day 97), seedlings inoculated with the AM fungus and grown under WW had

(SPAD-502, Minolta, Tokyo, Japan).

Chl fluorescence parameters were measured with a modulated Chl fluorometer (*MINI-Imaging-PAM*, Walz, Germany) at room temperature. The fifth fully expanded leaf (from the apex) of each seedling was dark-adapted for 30 min. The F_0 (minimum fluorescence yield of dark-adapted leaves) was recorded. The F_m (maximal fluorescence yield of dark-adapted leaves) was determined after a saturating pulse of $3,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for 3 s. Then the leaves were placed under an active light of $300 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ to measure the F_m' (maximal fluorescence yield of light-adapted leaves), F_0' (minimum fluorescence yield of light-adapted leaves), and F_s (steady-state fluorescence of light-adapted leaves). The maximum quantum yield of PSII (F_v/F_m , where $F_v = F_m - F_0$) was calculated. The actual quantum yield of PSII [$\Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$], the photochemical quenching [$q_p = (F_m' - F_s)/(F_m' - F_0')$], and the nonphotochemical quenching [$q_N = 1 - (F_m' - F_0')/(F_m - F_0)$] were calculated in the light-adapted state.

Gas-exchange parameters: Net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were measured with a portable photosynthesis system *Li-6400* (*LiCor*, Lincoln, NE, USA). The measurements were made at a light intensity of $1,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ from 08:30 to 11:30 h. The flow rate through the sample chamber was set at 500mL s^{-1} , the CO_2 concentration in the sample chamber was $400 \mu\text{mol mol}^{-1}$, and the leaf temperature was $35 \pm 0.8^\circ\text{C}$. Water-use efficiency (WUE) was calculated as the ratio between P_N and E .

Statistical analysis: Experimental data were subjected to two-way analysis of variance (*ANOVA*) using *SPSS 17.0* statistical program (*SPSS Inc.*, IL, USA), and the means were compared by *Duncan's* tests at the 0.05 level of significance ($n = 6$).

significantly greater stem length (117.2%), leaf dry mass (31.8%), stem dry mass (53.4%), and root dry mass (36.8%) compared with the control seedlings (Tables 2, 3).

Table 1. Effect of arbuscular mycorrhizal (AM) fungus on the frequency of AM colonization of poplar seedlings measured at different stages of the experiment. *Different letters* within each line denote significant differences at $P < 0.05$. DS – drought stress; PS – prior stress; REC – recovery; WW – well-watered.

Treatments		AM colonization [%]		
		PS	DS	REC
Nonmycorrhiza	WW	0	0	0
	DS	0	0	0
Mycorrhiza	WW	85.6 ^a	86.2 ^a	87.1 ^a
	DS	85.6 ^a	87.3 ^a	87.9 ^a

Table 2. Effect of arbuscular mycorrhizal fungi treatment (AMF), drought stress (DS), and AMF × DS on the parameters of poplar seedlings at different experimental stages. PS – prior stress; REC – recovery; C_i – intercellular CO₂ concentration; E – transpiration rate; F_v/F_m – the maximal quantum yield of PSII in dark-adapted state; g_s – stomatal conductance; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; REC – recovery; RWC – relative water content; WUE – water-use efficiency; Φ_{PSII} – effective quantum yield of PSII. ** P <0.01; * P <0.05; ns – not significant.

Parameters	PS AMF	DS AMF	DS	AMF × DS	REC AMF	DS	AMF × DS
Stem length	**	**	**	ns	**	*	ns
Root dry mass	**	**	ns	ns	**	ns	ns
Stem dry mass	**	**	ns	ns	**	ns	ns
Leaf dry mass	**	**	ns	ns	**	ns	ns
RWC	ns	**	**	**	ns	*	ns
WUE	ns	**	ns	ns	**	**	ns
SPAD	*	*	ns	ns	**	ns	ns
F_v/F_m	**	**	**	ns	**	ns	ns
Φ_{PSII}	**	**	**	**	**	**	**
q_P	**	**	**	ns	**	ns	*
q_N	**	**	**	ns	**	ns	**
P_N	**	**	**	ns	**	ns	ns
g_s	ns	**	**	**	ns	**	ns
C_i	**	**	**	**	**	**	ns
E	**	**	**	ns	**	**	ns

Table 3. Effects of arbuscular mycorrhizal colonization on stem length and dry mass of poplar seedlings harvested at different experimental stages. Means ± SE ($n = 6$); different letters within each row denote significant differences at P <0.05. DS – drought stress; PS – prior stress; REC – recovery; WW – well-watered.

Parameters	Time	Nonmycorrhizal seedlings		Mycorrhizal seedlings		
		WW	DS	WW	DS	
Stem length [cm]	PS	27.75 ± 1.06 ^b	27.75 ± 1.06 ^b	59.33 ± 1.05 ^a	59.33 ± 1.05 ^a	
	DS	30.92 ± 0.61 ^c	28.43 ± 1.14 ^c	66.32 ± 1.10 ^a	62.37 ± 1.45 ^b	
	REC	41.33 ± 1.14 ^b	34.93 ± 1.59 ^c	77.83 ± 1.69 ^a	74.35 ± 2.41 ^a	
Dry mass [g·per pot]	Leaf	PS	3.61 ± 0.30 ^b	3.61 ± 0.30 ^b	4.90 ± 0.16 ^a	4.90 ± 0.16 ^a
		DS	3.74 ± 0.30 ^b	3.67 ± 0.26 ^b	4.93 ± 0.12 ^a	4.94 ± 0.06 ^a
		REC	4.04 ± 0.34 ^b	3.92 ± 0.25 ^b	5.58 ± 0.21 ^a	5.26 ± 0.26 ^a
	Stem	PS	1.08 ± 0.06 ^b	1.08 ± 0.06 ^b	1.64 ± 0.09 ^a	1.64 ± 0.09 ^a
		DS	1.16 ± 0.07 ^b	1.12 ± 0.10 ^b	1.78 ± 0.09 ^a	1.82 ± 0.05 ^a
		REC	1.23 ± 0.07 ^b	1.23 ± 0.06 ^b	2.29 ± 0.08 ^a	2.18 ± 0.15 ^a
	Root	PS	1.19 ± 0.09 ^b	1.19 ± 0.09 ^b	1.69 ± 0.10 ^a	1.69 ± 0.10 ^a
		DS	1.25 ± 0.06 ^b	1.21 ± 0.06 ^b	1.71 ± 0.08 ^a	1.77 ± 0.07 ^a
		REC	1.50 ± 0.05 ^b	1.46 ± 0.06 ^b	2.03 ± 0.11 ^a	2.05 ± 0.17 ^a

Under DS, the stem lengths and dry masses of the leaves, stems, and roots of the mycorrhizal seedlings were 119.3, 34.6, 62.5, and 46.3% greater, respectively, than those of the nonmycorrhizal control seedlings.

By the end of the REC stage (day 127), the seedlings inoculated with *R. irregularis* showed visibly longer stem lengths and greater leaf, stem, and root dry masses under both watering regimes compared with the nonmycorrhizal control seedlings (Tables 2, 3).

Water status and Chl content: Measurements taken at the end of the PS stage revealed that AM symbiosis had

a significant positive effect on the SPAD value, but no effect on the RWC or on the WUE of poplar seedlings (Tables 2, 4). The SPAD value of mycorrhizal seedlings was 7.4% higher than that of nonmycorrhizal seedlings (Table 4).

By the end of the DS stage, the SPAD value and the WUE of seedlings that had received the AM fungal treatment were significantly different from those of the control seedlings (Table 2). The RWC was markedly affected by the AM fungal treatment, DS, and by the combination of the AM fungal treatment and DS. Inoculation of poplar seedlings with the AM fungus had a

Table 4. Effects of arbuscular mycorrhizal colonization on SPAD value, relative water content (RWC), and instantaneous water-use efficiency (WUE) of poplar seedlings at different experimental stages. Means \pm SE ($n = 6$); *different letters* within each row denote significant differences at $P < 0.05$. DS – drought stress; PS – prior stress; REC – recovery; WW – well-watered.

Parameters	Time	Nonmycorrhizal seedlings		Mycorrhizal seedlings	
		WW	DS	WW	DS
SPAD [arbitrary units]	PS	37.32 \pm 0.67 ^b	37.32 \pm 0.67 ^b	40.10 \pm 0.83 ^a	40.10 \pm 0.83 ^a
	DS	38.17 \pm 0.42 ^b	39.33 \pm 1.03 ^{ab}	40.93 \pm 0.43 ^a	39.93 \pm 0.75 ^{ab}
	REC	37.45 \pm 0.35 ^b	40.00 \pm 1.09 ^a	41.05 \pm 0.51 ^a	41.22 \pm 0.72 ^a
RWC [%]	PS	93.62 \pm 1.49 ^a	93.62 \pm 1.49 ^a	96.17 \pm 0.86 ^a	96.17 \pm 0.86 ^a
	DS	94.93 \pm 0.91 ^a	80.63 \pm 0.85 ^c	95.68 \pm 0.89 ^a	88.87 \pm 2.15 ^b
	REC	94.08 \pm 1.28 ^{ab}	91.64 \pm 1.30 ^b	95.99 \pm 0.78 ^a	92.85 \pm 1.00 ^{ab}
WUE [$\mu\text{mol}(\text{CO}_2) \text{mmol}^{-1}(\text{H}_2\text{O})$]	PS	2.88 \pm 0.10 ^a	2.88 \pm 0.10 ^a	3.15 \pm 0.08 ^a	3.15 \pm 0.08 ^a
	DS	2.93 \pm 0.06 ^b	3.01 \pm 0.18 ^b	3.18 \pm 0.17 ^b	3.59 \pm 0.11 ^a
	REC	2.95 \pm 0.06 ^c	3.15 \pm 0.04 ^{bc}	3.31 \pm 0.15 ^{ab}	3.55 \pm 0.06 ^a

positive effect on the SPAD value of the seedlings under WW conditions, but no effect under DS (Table 4). The RWC and WUE of mycorrhizal seedlings were 10.2 and 19.3% higher, respectively, than nonmycorrhizal seedlings under DS, whereas no differences were found under WW conditions.

Measurements taken at the end of the REC stage showed that AM fungus inoculation influenced the SPAD value and WUE markedly and DS significantly affected the RWC and WUE (Table 2). Inoculation with the AM fungus had a positive effect on the SPAD value under WW conditions, but no effect under DS (Table 4). Regardless of the watering regime, mycorrhizal poplars showed higher RWC than nonmycorrhizal seedlings, although the difference was not significant. Mycorrhizal seedlings had higher WUE than nonmycorrhizal seedlings under both watering regimes.

Chl fluorescence parameters: By the end of the PS stage, seedlings with AM fungal treatment exhibited significantly greater F_v/F_m (3.8%), Φ_{PSII} (28.9%), q_p (5.9%), and q_N (20.9%) compared with the nonmycorrhizal control seedlings (Table 2, Fig. 1).

By the end of the stressed stage, the F_v/F_m , Φ_{PSII} , q_p , and q_N of poplar seedlings were markedly affected by AM fungal treatment and DS; however, only the Φ_{PSII} was significantly affected by the combination of AM treatment and DS (Table 2). Compared with nonmycorrhizal poplars, the F_v/F_m , Φ_{PSII} , q_p , and q_N of mycorrhizal seedlings were 3.8, 26.0, 7.3, and 17.9% higher, respectively, under WW conditions, and 5.2, 4.9, 11.4, and 14.2% higher, respectively, under DS conditions. Therefore, under DS conditions, the F_v/F_m , Φ_{PSII} , q_p , and q_N of nonmycorrhizal seedlings decreased by 6.5, 9.6, 10.8, and 13.5%, respectively, and by 5.2, 24.7, 7.3, and 16.2%, respectively, in mycorrhizal seedlings (Fig. 1).

By the end of the REC stage, the mycorrhizal treatment significantly influenced the F_v/F_m , Φ_{PSII} , q_p , and q_N of poplar seedlings; only the Φ_{PSII} was affected by DS; the

combination of mycorrhizal treatment and DS affected the Φ_{PSII} , q_p , and q_N (Table 2). Compared with the nonmycorrhizal seedlings, the F_v/F_m , Φ_{PSII} , q_p , and q_N of mycorrhizal seedlings under WW conditions were 3.0, 29.0, 6.1, and 19.8% higher, respectively, and 5.6, 9.3, 11.0, and 9.6% higher, respectively, under DS (Fig. 1).

Gas exchange: By the end of the PS stage, the mycorrhizal treatment showed a significant positive effect on the P_N and E , which were 34.3 and 22.7% higher, respectively, than that of the nonmycorrhizal seedlings. However, the mycorrhizal treatment had an obvious negative effect on the C_i (5.4% lower than that of the nonmycorrhizal seedlings), and no effect on the g_s (Table 2, Fig. 2).

By the end of the DS stage, the P_N , g_s , C_i , and E of seedlings with the mycorrhizal treatment or the DS treatment were markedly affected; however, only the g_s and C_i were significantly affected by the combination of mycorrhizal treatment and the DS treatment (Table 2). The P_N , g_s , and E declined under DS conditions regardless of whether the seedlings had been inoculated with the AM fungus (Fig. 2). The AM fungal treatment had a positive effect on the P_N , g_s , and E (29.3, 5.2, 20.3% higher, respectively, under WW conditions, and 54.9, 84.6, 28.6% higher, respectively, under DS conditions). However, the AM fungal treatment had a negative effect on the C_i of seedlings (6.2% lower under WW conditions and 2.7% lower under DS conditions).

By the end of the REC stage, the P_N , C_i , and E of seedlings with the mycorrhizal treatment were significantly different from that of the nonmycorrhizal control seedlings. The DS treatment affected the g_s , C_i , and E (Table 2). The P_N , g_s , and E of WW-treated mycorrhizal seedlings were 29.6, 3.5, and 24.1% higher, respectively, whereas the C_i was 5.7% lower than that of the nonmycorrhizal seedlings (Fig. 2). The P_N , g_s , and E of the mycorrhizal seedlings that received the DS treatment were 20.0, 8.2, and 11.7% higher, respectively, whereas the C_i was 5.5% lower than that of the nonmycorrhizal seedlings.

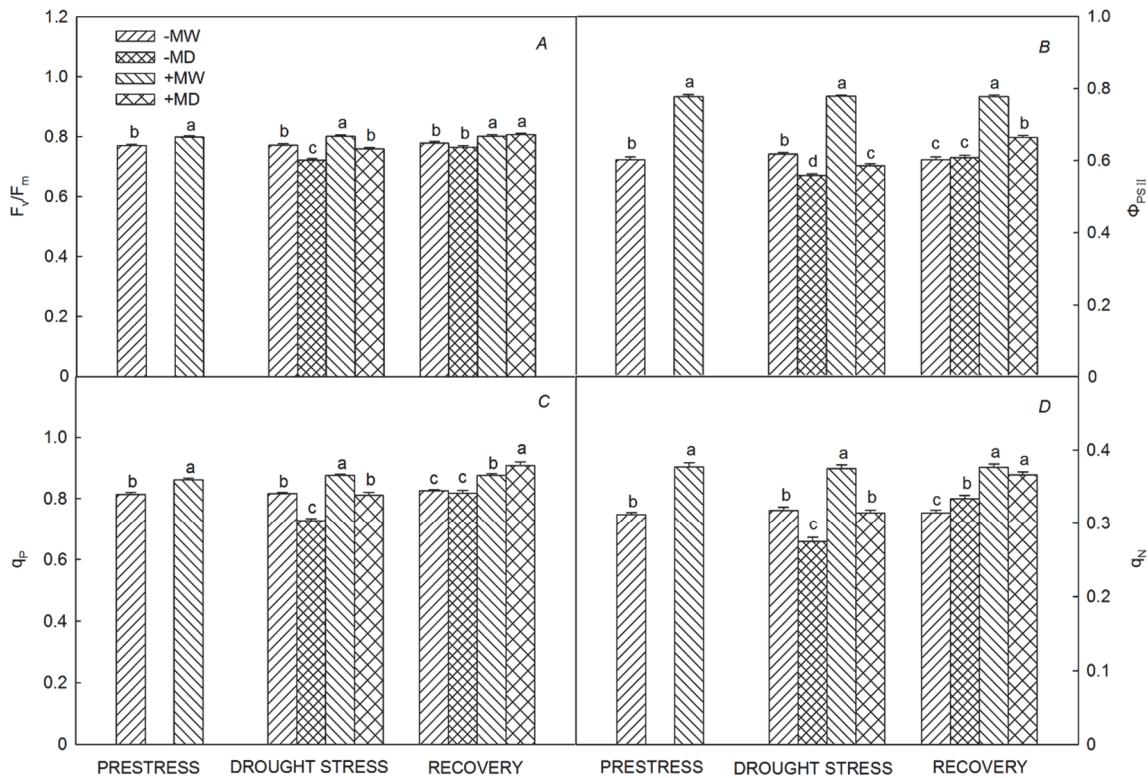


Fig. 1. Effects of the arbuscular mycorrhizal fungus on the maximal quantum yield of PSII in dark-adapted state (F_v/F_m) (A), effective quantum yield of PSII (Φ_{PSII}) (B), photochemical quenching (q_p) (C), and nonphotochemical quenching (q_N) (D) of poplar seedlings at different experimental stages. The mean \pm SE ($n = 6$). Different letters above the error bars indicate significant differences at $P < 0.05$ at each experimental stage. -MW – nonmycorrhizal seedlings and well watered; -MD – nonmycorrhizal seedlings and drought stressed; +MW – mycorrhizal seedlings and well watered; +MD – mycorrhizal seedlings and drought stressed.

Discussion

Previous studies in strawberry (*Fragaria virginiana*), sunflower (*Helianthus annuus*), and *Sophora davidii* have reported that drought stress had a negative effect on mycorrhizal colonization (Borowicz 2010, Gholamhoseini *et al.* 2013, Gong *et al.* 2013), mainly because drought stress inhibited spore germination and hyphal growth (Huang *et al.* 2011, Gong *et al.* 2013). The frequency of AM colonization can indicate the extent of AM fungi hypha colonized the plant root. In this study, the frequency of AM colonization was determined. However, the data showed that the frequency of AM colonization of poplars was not affected by drought stress. We hypothesized that once the frequency of mycorrhizal colonization reached a certain extent it was not easy to affect it by a short-term stress. Another possible reason was that the drought stress was not severe or long enough to impact it in this experiment.

Previous studies using *S. davidii* and maize have shown that the biomass of host plants inoculated with AM fungi was higher than that of nonmycorrhizal plants (Sheng *et al.* 2008, Gong *et al.* 2013). Our data showed that mycorrhizal

poplar seedlings accumulated more biomass during the PS, DS, and REC stages compared with the nonmycorrhizal ones. DS did not influence the biomass significantly. The reason might be that biomass accumulation is a long-term process for plant, thus the short-term drought period may not greatly influence the biomass. Furthermore, mycorrhizal poplars accumulated more biomass under DS than under WW conditions compared with the nonmycorrhizal poplars. This suggests that AM fungi performed a more important role under DS than under WW conditions. The results also showed that DS negatively affected the stem length of nonmycorrhizal seedlings which was evident in the REC stage by a reduced growth. On the other hand, mycorrhizal seedlings quickly recovered during REC stage. This implied that DS caused damage to nonmycorrhizal seedlings. Although DS inhibited the growth of mycorrhizal seedlings when they suffered stress, they recovered rapidly to the same level as the WW seedlings.

Plant water relations regulate gas-exchange parameters, such as g_s and E (e.g. Zhu *et al.* 2012). An important

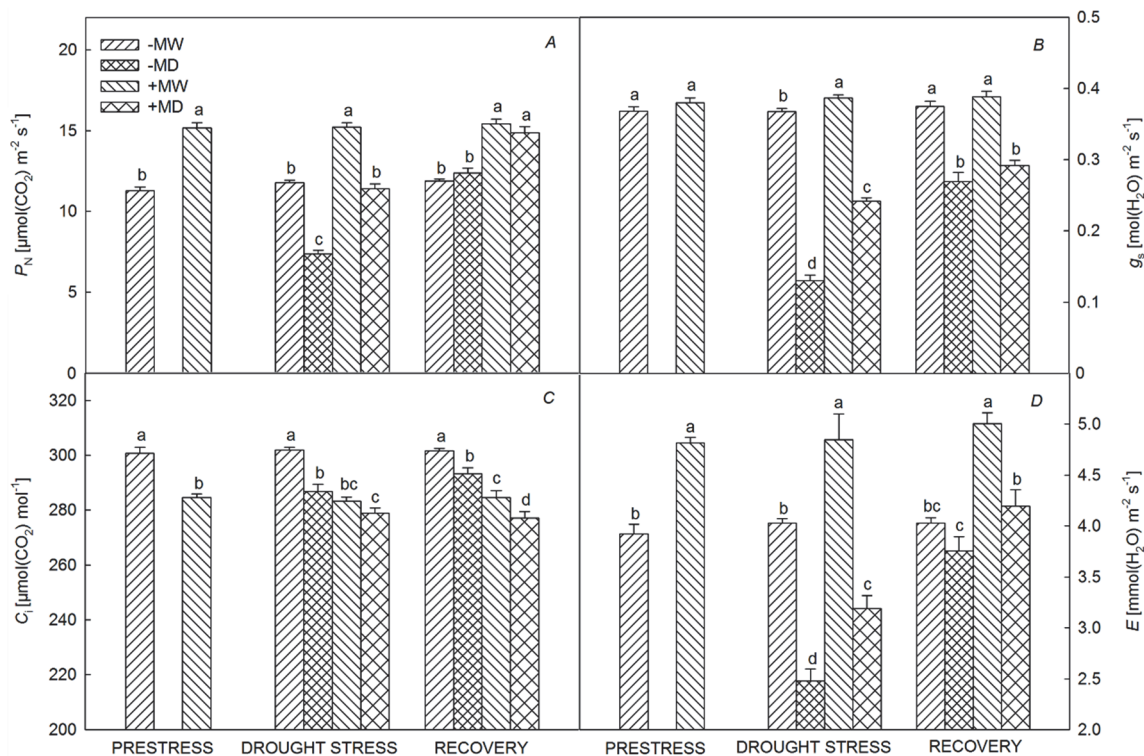


Fig. 2. Effects of arbuscular mycorrhizal fungus on the net photosynthetic rate (P_N) (A), stomatal conductance (g_s) (B), intercellular CO_2 concentration (C_i) (C), and transpiration rate (E) (D) of poplar seedlings at different experimental stages. The means \pm SE ($n = 6$). Different letters above the error bars indicate significant differences at $P < 0.05$ at each experimental stage. –MW – nonmycorrhizal seedlings and well watered; –MD – nonmycorrhizal seedlings and drought stressed; +MW – mycorrhizal seedlings and well watered; +MD – mycorrhizal seedlings and drought stressed.

mechanism, by which plants avoid drought stress, is to modify their root system to absorb more water (Songsri *et al.* 2009). Previous studies have found that mycorrhizal plants often show higher leaf RWC and WUE compared to nonmycorrhizal plants. AM fungi can be beneficial for water transportation through plants and can help plants to keep stomata open (Nelson and Safir 1982, Zhu *et al.* 2012). In this study, the RWC and WUE in the mycorrhizal and nonmycorrhizal poplars were not significantly different when the seedlings were grown under WW conditions. But AM fungus significantly increased the RWC and WUE under DS condition. DS decreased the RWC of both mycorrhizal and nonmycorrhizal seedlings, and increased the WUE of mycorrhizal seedlings. Our results were in agreement with Gong *et al.* (2013), who also showed that mycorrhizal seedlings improved their water uptake compared to nonmycorrhizal seedlings under drought stress condition. It is likely that the hyphae of the AM fungus expanded the absorption region of the host plant roots (Muthukumar and Udaiyan 2010), and enhanced the absorption of water by the roots (Huang *et al.* 2011). Moreover, better water status might result in the increased activity and hydraulic conductivity of the roots (Zhu *et al.* 2010). During the recovery period, mycorrhizal poplars still maintained a higher water status to remedy the injury that had been caused by the DS.

Our results support previous findings that AM fungi inoculation enhances the Chl content of their host plant (Paradis *et al.* 1995, Sannazzaro *et al.* 2006, Colla *et al.* 2008, Sheng *et al.* 2008). However, the enhancement of Chl content in mycorrhizal seedlings has not been linked to drought resistance. The light energy absorbed by Chl molecules in plant leaves is either used to drive photosynthesis (photochemistry), or is emitted as Chl fluorescence, or is dissipated as heat (Sheng *et al.* 2008), depending on the biochemical and environmental conditions (Qiu *et al.* 2012). Chl fluorescence can reflect the photosynthetic activity of the leaves in an intricate way, and it is often used to analyze the photosynthesis and related mechanisms in a plant that has suffered biotic or abiotic stress (Qiu *et al.* 2012). The ratio of F_v/F_m can be used to measure the capacity of the primary photochemistry of PSII (Sheng *et al.* 2008). Our data support findings of previous studies showing that AM symbiosis improved the F_v/F_m in the leaves of maize (Sheng *et al.* 2008, Zhu *et al.* 2012). We found that the AM fungus used in this study increased the drought tolerance of poplar by reducing the decline in F_v/F_m and q_p caused by drought stress. Throughout the experiment, mycorrhizal poplars showed higher F_v/F_m , Φ_{PSII} , q_p , and q_N than nonmycorrhizal poplars. This indicates that an AM symbiotic relationship can enhance the efficiency of excitation energy capture by

chloroplasts, increase the photochemical capacity of PSII in light-adapted leaves (Gong *et al.* 2013).

Photosynthesis is an important physiological process for biomass accumulation (Qiu *et al.* 2012). Our results showed that mycorrhizal plants had higher P_N and E but lower C_i , compared with nonmycorrhizal plants, which supports previous mycorrhizal studies using *S. davidii*, watermelon (*Citrullus lanatus* Thunb.), citrus (*Citrus tangerine* Hort. ex Tanaka), and maize (Kaya *et al.* 2003, Sheng *et al.* 2008, Wu *et al.* 2010, Gong *et al.* 2013). We also found that the extent to which P_N , g_s , and E decreased, when the seedlings were grown under DS conditions, was lower in the mycorrhizal than in nonmycorrhizal seedlings. Therefore the AM symbiotic relationship seems to be more important for maintaining the P_N of poplars under DS than under WW conditions. This suggests that AM fungi might

act as an important stress buffer when their host plants suffer an adverse stress (Sheng *et al.* 2008). Our study showed that mycorrhizal poplars had higher photosynthetic capacity than nonmycorrhizal poplars throughout the experiment, which might reduce the biomass loss caused by periods of drought, and be beneficial to biomass production.

In conclusion, higher Chl content and Chl fluorescence parameters mean higher photosynthetic capacity and, hence, greater biomass accumulation. Mycorrhizal poplars had higher Chl content, Chl fluorescence parameters, and gas-exchange capacity and were therefore able to grow well even under drought stress conditions. AM fungi might stimulate the hosts to use water more efficiently under drought stress conditions.

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