Arbuscular mycorrhizae improves photosynthesis and water status of *Zea mays* L. under drought stress

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ABSTRACT

The influences of arbuscular mycorrhizal (AM) fungus on growth, gas exchange, chlorophyll concentration, chlorophyll fluorescence and water status of maize (*Zea mays* L.) plants were studied in pot culture under well-watered and drought stress conditions. The maize plants were grown in a sand and black soil mixture for 4 weeks, and then exposed to drought stress for 4 weeks. Drought stress significantly decreased AM colonization and total dry weight. AM symbioses notably enhanced net photosynthetic rate and transpiration rate, but decreased intercellular CO_2 concentration of maize plants regardless of water treatments. Mycorrhizal plants had higher stomatal conductance than non-mycorrhizal plants under drought stress. The concentrations of chlorophyll were higher in mycorrhizal than non-mycorrhizal plants under drought stress. AM colonization significantly increased maximal fluorescence, maximum quantum efficiency of PSII photochemistry and potential photochemical efficiency, but decreased primary fluorescence under well-watered and droughted conditions. Mycorrhizal maize plants had higher relative water content and water use efficiency under drought stress compared with non-mycorrhizal plants. The results indicated that AM symbiosis alleviates the toxic effect of drought stress via improving photosynthesis and water status of maize plants.

Keywords: chlorophyll concentration; chlorophyll fluorescence; gas exchange; maize; water status

Plant is constantly faced with environmental stress factors of both biotic and abiotic origin. Drought stress is one of the most important abiotic stress factors that limits plant growth and development in many arid and semiarid regions of the world, and seasonal drought often occurs in the non-arid regions. Drought caused a great reduction in productivity and yield of crop, although crop characteristics were associated with improved capability under drought stress which allows crops to absorb more water, to reduce water loss rates, and to maintain higher physiological activity. In addition, drought caused by climate change and water shortages will become more serious, which will drastically affect growth and survival of plants and crops in many parts of the world.

Arbuscular mycorrhizal (AM) symbiosis is formed between the majority (70–90%) of terrestrial plants and fungi of the monophyletic phylum Glomeromycota (Schüssler et al. 2001). AM fungi are known to improve the mineral nutrition of colonized plants, they also increase their resistance to drastic environmental conditions, especially drought stress (Smith and Read 2008).

It has been demonstrated that AM symbiosis affects the water relations of many plants. The AM symbiosis often results in the change of water movement into, through, and out of the host plants, with consequent effects on tissue hydration and plant physiology. The contribution of mycorrhizal symbiosis to drought tolerance is due to a combination of physical, nutritional, physiological, and cellular effects (Aroca et al. 2008, Miransari 2010). AM and non-AM plants often show different photosynthetic characters. Wu and Xia (2006) confirmed that AM citrus seedlings had higher

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photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) than corresponding non-AM plants under drought stress. It is known that the concentration of chlorophyll is associated with P_n, and the characterization of chlorophyll fluorescence reflects the state of the photosynthetic apparatus. However, the knowledge about chlorophyll concentration and chlorophyll fluorescence of AM plants under drought stress are insufficient.

Hence, the objectives of this study were to evaluate the effects of AM fungus, *Glomus etunicatum*, on growth, gas exchange, chlorophyll concentrations, chlorophyll fluorescence and water status of maize plants under drought stress, and to analyze the correlation between water status and photosynthesis, so as to understand the drought tolerance mechanism of AM plants.

MATERIAL AND METHODS

Soil and biological materials. Seeds of maize genotype Zhengdan 958 were surface sterilized with 0.5% NaClO for 30 min, washed four times with sterile distilled water, and then germinated on moist filter paper in Petri dishes at 28°C. Three pre-germinated seeds were sown in pots containing 2.8 kg of the black soil and sand mixture. Black soil was collected from Dehui City, Jilin Province, China, sieved (2 mm), mixed with sand (1:1.5, soil:sand, v/v) and sterilized by steaming at 100°C for 1 h on three consecutive days. The soil properties were 26.9 g/kg organic matter, 118.8 mg/kg available nitrogen, 18 mg/kg available phosphorus, 111 mg/kg available potassium and pH 6.6. Seedlings were thinned to two seedlings per pot 1 week after emergence.

The AM fungus inoculums were provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, China. The inoculums consisted of soil, spores (the spores density was 3000 per 20 mL inoculums), mycelium and infected jowar root fragment from a stock culture of *G. etunicatum*. The 25 g inoculum for the mycorrhizal treatment was placed 4 cm below the maize seeds at sowing time.

Experimental design. The experimental treatments were two soil water regimes (well-watered control and water stress) and two mycorrhizal inoculums (non-mycorrhizal control and *G. etunicatum*). The experiment was a randomized blocks design with three replicates. Maize seedlings were grown in a greenhouse at 20–28°C with 14 h day light and 75–90% relative humidity. Water was supplied daily to maintain 75% of relative soil water content by weighting the substrate before and after drying at 105°C for 24 h during the first 4 weeks of plant growth. At this time half of the plants were exposed to drought stress (55% of relative soil water content) for 4 weeks. The water status in the substrate was daily determined and the amount of water loss was supplied to each pot to keep the designed soil water contents.

Measurements. Four weeks after treatment, the plant height was recorded. The root, shoot and total dry weights were determined after drying at 75°C for 48 h. The AM colonization was measured according to the gridline intercept method described by Giovannetti and Mosse (1980). Gas exchange parameters including P_n, E, g_s, and intercellular CO_2 concentration (C_i) were measured with a portable open flow gas exchange system LI-6400 (LI-COR, Lincoln, USA) from 09:00 to 12:00 am. The concentrations of chlorophyll *a*, *b*, and *a* + *b* was calculated according to Zhang and Zhang (2006). Chlorophyll fluorescence parameters were measured at room temperature using a portable fluorometer OS-30P (OPTI, Boston, USA) according to the manufacturer's instructions. Relative water content (RWC) was measured by Zhang and Zhang (2006). Water use efficiency (WUE) was determined by the ratio of P_n to E.

Statistical analysis. The experimental data were analysed statistically by two-way ANOVA with SPSS 13.0 software and treatment means were compared by the Duncan's test ($P < 0.05$).

RESULTS AND DISCUSSION

The roots of non-inoculated maize plants were not colonized by AM fungus. The plants inoculated with AM fungus were infected under well-watered and drought stress (Table 1), and drought stress significantly decreased AM colonization.

It is currently accepted that AM symbiosis affects plant growth and biomass. In this study, plant height, shoot dry weight and total dry weight were significantly lower for drought-stressed maize plants than those for well-watered plants (Table 1). However, the root dry weight of all maize plants was similar regardless of water treatments. The differences of plant height, shoot dry weight, root dry weight and total dry weight were not significant between mycorrhizal and non-mycorrhizal plants. Similar results were reported by some literature (Porcel and Ruiz-Lozano 2004, Kohler et al. 2008).

Table 1. AM root colonization, plant height and dry weight of maize plants inoculated (M+) or not (M–) with *Glomus etunicatum* under well-watered and drought-stressed conditions

Water regime	Inoculation	Root colonization (%)	Plant height (cm)	Dry weight (g/plant)		
				shoot	root	total
Well-watered	M+	41.7[a]	91.2[a]	2.7[a]	0.4[a]	3.1[a]
	M–	–	89.2[a]	2.5[a]	0.4[a]	2.9[a]
Drought-stressed	M+	33.0[b]	82.7[ab]	1.8[b]	0.3[a]	2.0[b]
	M–	–	74.6[b]	1.4[b]	0.2[a]	1.6[b]

Means followed by the same letter are not significantly different ($P < 0.05$)

AM colonization did not result in higher plant height and dry weight. This observation may be related to carbon drain effect or dysfunction of maize metabolism (Liu et al. 2004).

The rate of gas exchange is an important factor that influences plant growth under drought stress. Mycorrhizal plants often display higher P_n than non-mycorrhizal plants, which is consistent with AM symbioses effects on g_s (cf. Augé 2001). In the present study, mycorrhizal maize plants had higher P_n and *E* than non-mycorrhizal plants under well-watered and drought stress conditions (Figure 1). The g_s in AM-inoculated plants was significantly higher than in non-inoculated plants under drought stress. Compared with non-mycorrhizal plants, g_s was 24.6% higher in mycorrhizal plants. The results implied that AM symbioses could enhance the gas exchange capacity via decreased stomatal resistances and increased transpiration fluxes. The lower values of stomatal resistance in mycorrhizal

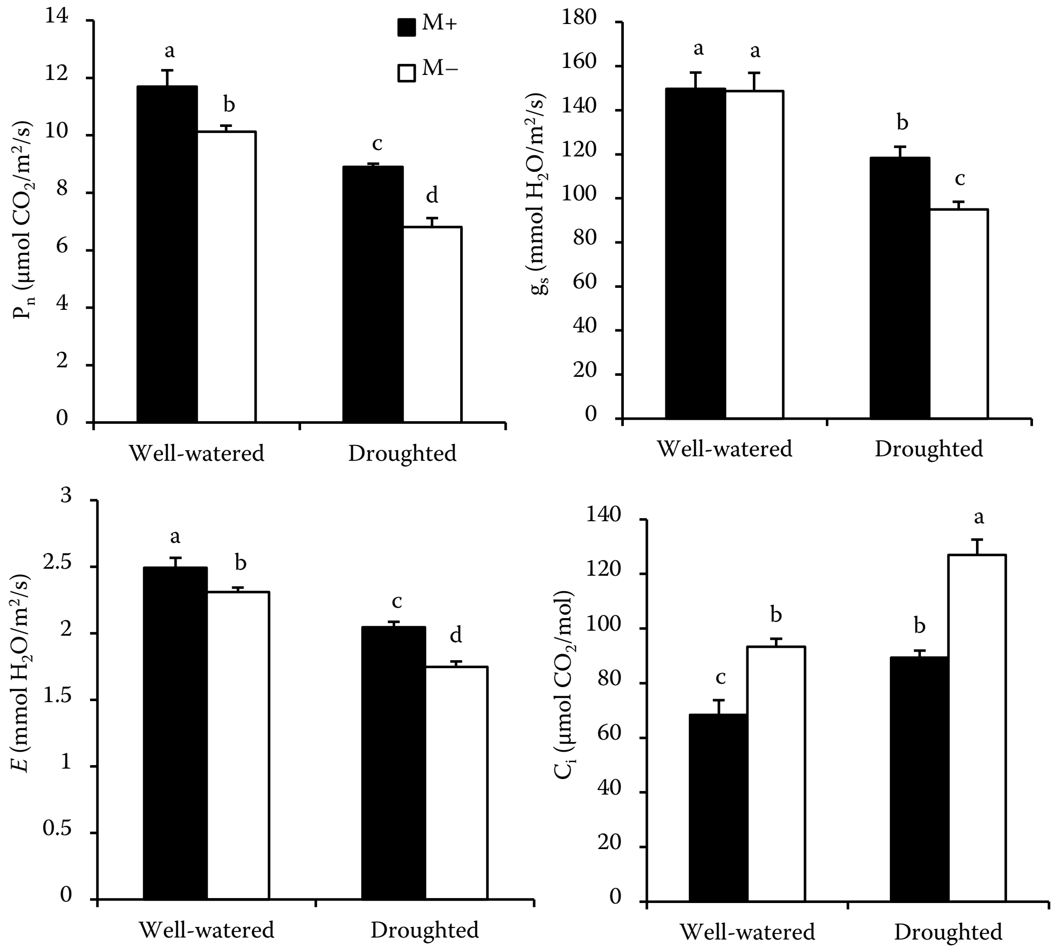

Figure 1. Net photosynthetic rate (P_n), transpiration rate (*E*), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) of maize plants inoculated (M+) or not (M–) with *Glomus etunicatum* under well-watered and drought stressed conditions. Mean pairs followed by different letters are significantly different ($P < 0.05$)

Table 2. Chlorophyll concentration (mg/g), primary fluorescence (F_o), maximal fluorescence (F_m) of maize plants inoculated (M+) or not (M–) with *Glomus etunicatum* under well-watered and drought-stressed conditions

Water regime	Inoculation	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a* + *b*	F_o	F_m
Well-watered	M+	3.1^{a}	0.9^{a}	3.9^{a}	75.7^{c}	373.4^{a}
	M–	2.7^{ab}	0.7^{ab}	3.5^{ab}	76.6^{bc}	355.0^{b}
Drought-stressed	M+	3.0^{a}	0.8^{a}	3.8^{a}	77.7^{b}	337.3^{c}
	M–	2.5^{b}	0.7^{b}	3.2^{b}	79.3^{a}	316.3^{d}

Means followed by the same letter are not significantly different ($P < 0.05$)

plants indicate that these plants were able to keep the stomata open longer than non-mycorrhizal plants (Subramanian et al. 1995). Some studied suggested that AM colonization may increase the number of photosynthetic units, and the rates of photosynthetic storage and export (cf. Augé 2001).

Chlorophyll status is a key index for evaluating plant photosynthetic efficiency and environmental stress. The concentrations of chlorophyll *a*, chlorophyll *b* and chlorophyll *a* + *b* were similar in mycorrhizal and non-mycorrhizal maize plants under well-watered conditions (Table 2). However, the differences of chlorophyll *a*, chlorophyll *b* and chlorophyll *a* + *b* concentrations were significant between AM and non-AM maize plants under drought stress conditions, which is in agreement with the results of other studies (cf. Augé 2001, Gemma et al. 1997). Compared with non-mycorrhizal plants, the concentrations of chlorophyll *a*, chlorophyll *b* and chlorophyll *a* + *b* of mycorrhizal plants were 18.6%, 27.5% and 20.5% higher under drought stress, respectively. This suggests that drought stress interferes less with chlorophyll synthesis and/or more with chlorophyll breakdown in mycorrhizal than in non-mycorrhizal plants (Evelin et al. 2009).

Chlorophyll fluorescence was proven to be a very useful noninvasive tool for the evaluation of environmental stresses effect on photosynthetic properties. In the present study, the primary fluorescence (F_o) of mycorrhizal plants was lower than that non-mycorrhizal plants under drought stress (Table 2). Mycorrhizal plants had markedly higher maximal fluorescence (F_m) than non-mycorrhizal plants regardless of water treatments. Drought stress increased F_o, but decreased F_m of both AM-inoculated and non-inoculated plants. The results indicated that drought stress destructs the structure and function of PSII reaction center and disrupts electron transport in photosynthetic apparatus, whereas AM symbiosis mitigates the adverse influence of drought stress on PSII reaction center and photosynthetic efficiency (Baker 2008).

The ratio of F_v/F_m is a useful relative measure of the maximum quantum efficiency of PSII photochemistry, which provides a simple and rapid way of monitoring environmental stress (Krause and Weis 1991, Baker 2008). In this study, the F_v/F_m in mycorrhizal maize plants was higher than in non-mycorrhizal plants regardless of water treatments, and drought stress drastically decreased the ratio of F_v/F_m (Figure 2), which is in agreement with the

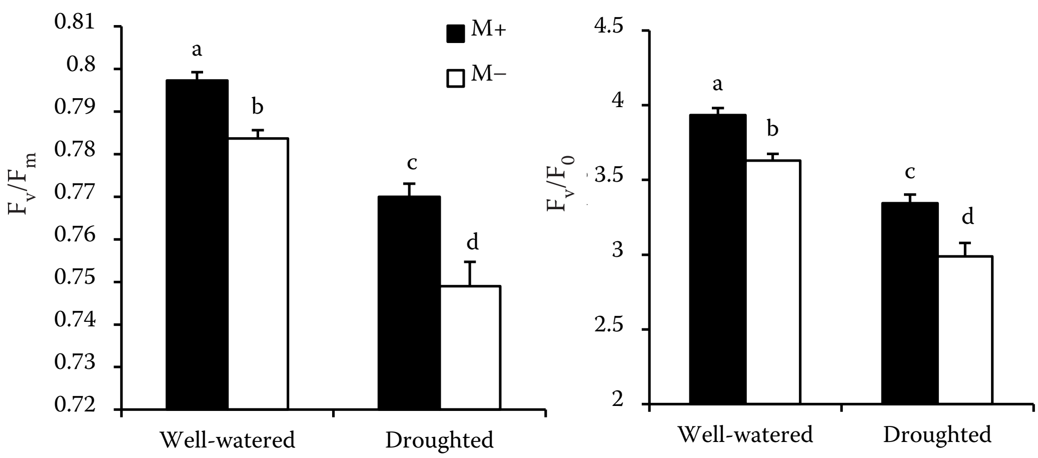

Figure 2. Maximum quantum efficiency of PSII (F_v/F_m) and potential photochemical efficiency (F_v/F_o) of maize plants inoculated (M+) or not (M–) with *Glomus etunicatum* under well-watered and drought stressed conditions. Mean pairs followed by different letters are significantly different ($P < 0.05$)

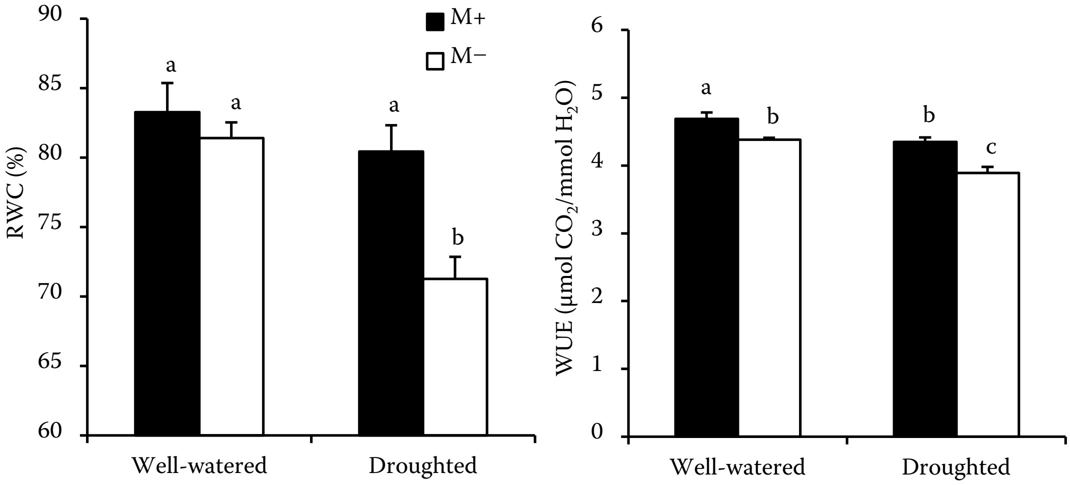

Figure 3. Relative water content (RWC) and water use efficiency (WUE) of maize plants inoculated (M+) or not (M–) with *Glomus etunicatum* under well-watered and drought stressed conditions. Mean pairs followed by different letters are significantly different (P <0.05)

results by Borkowska (2002). At the same time, the parameter F_v/F_o, an alternative expression of F_v/F_m, is a more sensitive and better value than F_v/F_m (Maxwell and Johnson 2000), also higher in mycorrhizal plants. These results indicated that AM symbioses might improve photochemistry efficiency of PSII under well-watered and droughted conditions.

It is well documented that AM symbiosis can improve the water status of host plant. Porcel and Ruiz-Lozano (2004) reported that mycorrhizal plants had higher leaf water potential compared with non-mycorrhizal plants under drought stress. Our study showed that AM symbiosis enhanced WUE (by 7.0% and 11.8%) in maize plant leaves under well-watered and droughted conditions (Figure 3). Drought stress significantly decreased WUE in both mycorrhizal and non-mycorrhizal plants. AM colonization also affected RWC of maize plants. Mycorrhizal plants had higher RWC than non-mycorrhizal plants under drought stress, but there was no difference between mycorrhizal and non-mycorrhizal plants under well-watered conditions. Drought stress markedly decreased RWC of non-mycorrhizal plants, but caused no significant difference between well-watered and droughted conditions for mycorrhizal plants. Mycorrhizal plants had better water status may due to external hyphal extraction of soil water (Ruiz-Lozano et al. 1995), stomatal regulation through hormonal signals (Aroca et al. 2008), indirect effect of improved phosphate and other nutrient uptake (Subramanian and Charest 1997), greater osmotic adjustment (Wu and Xia 2006) and higher root hydraulic conductivity (Augé 2004) than non-mycorrhizal plants. However, the RWC in mycorrhizal and non-mycorrhizal plants was similar under well-watered conditions, which indicated that leaf RWC of non-stressed plants were not affected by AM symbiosis.

Water status of mycorrhizal plants was often associtied with gas exchange, such as stomatal behavior and transpiration fluxes. Augé (2001) pointed out that g_s and leaf water potential are linked functionally: changes in one usually drive changes in the other. Higher RWC and WUE in mycorrhizal than in non-mycorrhizal plants may be beneficial for moving water through the plants to the evaporating surfaces and maintaining opened stomata in leaves (Nelsen and Safir 1982). Similarly, higher g_s and E in mycorrhizal compared to non-mycorrhzial plants may indicate lower resistance to water vapor transfer from inside the leaves to the atmosphere, which allows leaves to maintain more normal water balance (Augé 2004, Wu and Xia 2006). Thus, the results suggested that AM symbiosis protects maize plants against drought stress through improving gas exchange capacity, chlorophyll concentration, chlorophyll fluorescence and water status.

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