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ARBUSCULAR MYCORRHIZAL FUNGUS MODULATES VULNERABILITY TO XYLEM CAVITATION OF *POPULUS* × *CANADENSIS* ‘NEVA’ UNDER DROUGHT STRESS

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Abstract

To avoid xylem cavitation, plants adopt water manage behaviors from water-conserving to risk-taking to regulate stomatal conductance under drought stress, and the behaviors may shift due to sink demand. Arbuscular mycorrhizal (AM) fungi form symbioses with plants, generate sink demand and promote stomatal conductance, which would increase water loss and vulnerability to xylem cavitation. However, the effect of AM fungi on plant water manage behavior and vulnerability to xylem cavitation was rarely reported. The impact of an AM fungus (*Rhizophagus irregularis*) on growth, gas exchange, photosynthesis, and vulnerability to xylem cavitation of *Populus* × *canadensis* ‘Neva’ was evaluated under three water status (70%-75%, 50%-55%, 30%-35% of field capacity). After 250 days, *R. irregularis* colonized more than 70% roots, increased biomass accumulation, improved gas exchange, photosynthesis, leaf relative water content, and specific leaf area of poplar plants. Drought stress limited *R. irregularis* colonization rate, plant biomass accumulation, and parameters of gas exchange and photosynthesis. Inoculation of *R. irregularis* seemingly shifted water manage behavior from water-conserving toward risk-taking and modulated the vulnerability to xylem cavitation. Results of current study suggested that plant genes involved in water management and traits for xylem cavitation may be regulated by AM symbiosis and require further study.

Key words: Mycorrhizal symbiosis; Soil inoculant; Xylem cavitation; Mycoforestry.

Introduction

Drought, which limits plant growth and survival, is one of the most severe abiotic stresses. Based on physiological responds to drought, plants can be classified into isohydric and anisohydric species (Attia *et al*., 2015). Isohydric plants display water-conserving behavior to maintain a nearly constant leaf water potential, as they reduce stomatal conductance and transpiration. Meanwhile, anisohydric plants display risk-taking behavior, as they maintain stomatal conductance and transpiration and allow leaf water potential to drop (Sade & Moshelion, 2014). Under drought stress, a continuum was observed from isohydric species to anisohydric species for plants based on a meta-analysis (Klein, 2014). Moreover, the regulation of stomata of angiosperm may change along growing seasons and vary between isohydric and anisohydric behavior to acclimate environment. The behavior change may due to a function of sink demand (Sade & Moshelion, 2014).

Despite the plant behaviors, water loss via transpiration is an inevitable consequence of photosynthesis through stomata. Water loss through evaporation of leaf mesophyll cell walls generates tension to drive long-distance water transport in xylem conduits (Tyree & Zimmermann, 2002). When the xylem tension under drought stress overcomes the capillary forces holding water in the pit membrane pores, cavitation emerges and hydraulic conductivity and leaf gas exchange will be lowered (Fichot *et al*., 2015). Therefore, maintaining photosynthetic gas exchange and growth under drought stress requires avoidance of xylem cavitation. (Meinzer & McCulloh, 2013).

Arbuscular mycorrhizal (AM) fungi from Glomeromycota form symbiosis with plants and improve tolerance against abiotic stresses (Smith & Read, 2008; Bahaduiv *et al*., 2019). In the symbiosis, AM fungi enlarge carbon sink as they could consume up to 20% photosynthates of plants (Parniske, 2008). Moreover, plants form symbiosis with AM fungi usually had higher stomatal conductance than their non-mycorrhizal counterparts, and the promotion of stomatal conductance was intensified under drought stress (Augé *et al*., 2015). Higher sink demand may change the behavior of plants under drought stress, while higher stomatal conductance would generate higher water loss through evaporation and may intensify the vulnerability to xylem cavitation. However, the influence of AM fungus on plant behavior change and vulnerability to xylem cavitation was rarely reported.

Populus spp. is widely planted tree species for high economic value and ecological conservation (Eusemann *et al*., 2013; Zhou *et al*., 2020). The fast growing poplar species/genotypes consume a large amount of water, are relatively susceptible to drought stress and xylem cavitation, and generally considered to be isohydric plants (Arango-Velez *et al*., 2011; Cao *et al*., 2014; Attia *et al*., 2015). *Populus* × *canadensis* (a hybrid of *P. nigra* × *P. deltoides*) ‘Neva’ is widely planted in China (Liu *et al*., 2016), and this hybrid was used in current study to evaluate the influence of AM fungus on (1) the responding of poplar to intensified drought stress, and (2) the vulnerability to xylem cavitation.

Materials and Methods

Growth substrate, plant material, and AM fungal inoculation: The growth substrate and *Populus* × *canadensis* 'Neva' (15 cm in length) cuttings were prepared as described by Liu *et al*., (2016). Cuttings were planted in plastic pots (19.5 × 21.5 cm) filled with 5 kg growth substrate described above. One cutting was planted in each pot.

Inoculum of *Rhizophagus irregularis* was prepared and inoculated as described by Liu *et al*., (2016).

Experimental design and growth condition: The experiment consisted of a randomized block design with two factors (2 × 3): AM fungal inoculation (*R. irregularis* inoculated plants (AM) and non-inoculated plants (NM)) and water regimes (3 water conditions). Each treatment had 6 replicates. Pots were arranged in a randomized complete block design. After transplantation, cuttings were fertilized with 100 mL Hoagland's solution every 2 weeks and grown under well-watered condition. Sixty days post transplantation, plants in different water regimes received different water to match the well-watered condition (WW, 70%-75% of field capacity), mild stressed condition (MS, 50%-55% of field capacity), or severe stressed condition (SS, 30%-35% of field capacity). Field capacity of growth substrate was measured according to Bao (2000). Soil water content was controlled by weighing the pots every day. The water treatment was applied for 90 days

The pot experiment was conducted in a greenhouse of Northwest A&F University, Yangling under condition as described by Liu *et al*., (2016).

Plant biomass accumulation and mycorrhizal colonization: Two hundred and fifty days after transplantation, plants were harvested. Fresh weights of shoot and root were recorded. Mycorrhizal colonization was assessed by the gridline intercept method (Giovannetti & Mosse, 1980) after staining with trypan blue (Phillips & Heyman, 1970).

Gas exchange and relative chlorophyll content: The measurement of poplar leaf gas exchange parameters, which included net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (*C*i), and transpiration rate (*E*), and intrinsic water use efficiency (*WUEi*), were according to the description of Liu *et al*., (2016).

The leaves used for gas exchange measurement were also used for the relative chlorophyll content measurement. The relative chlorophyll content (soil and plant analyzer development (SPAD) value) was measured with a SPAD meter (SPAD-502 Plus, Konica-Minolta Holdings, Inc., Osaka, Japan).

Relative water content and specific leaf area measurement: Relative water content (RWC) was measured as described by Liu *et al*., (2016).

Specific leaf area (SLA) was calculated as the plant leaf area divided by the dry mass of leaf (Bao, 2000).

Xylem vulnerability curve: Before harvest, 3 shoot sections from each treatment were collected and cut into 27.4 cm fragment. The Cavitron technique was used for the assessment of vulnerability to xylem cavitation (Cochard *et al*., 2005). Xylem vulnerability curve was represented as the percentage loss of xylem hydraulic conductance (PCL) vs xylem water tension. Vulnerability to xylem cavitation was calculated as the water tension (P_{50}) that caused 50 % loss of xylem hydraulic conductance.

Data analysis

Statistical analyses were performed using the program package Statistica (Version 9.1; StatSoft Inc., Tulsa, OK, USA). The ANOVA was used to determine the effect of AM fungus and water regimes and their interactions. Fisher's LSD was performed at $P = 0.05$ in case of significant impact by factor. The Correlation analysis between xylem embolism vulnerability and plant growth parameters was carried out using Pearson' correlation coefficients.

Results

Biomass accumulation and mycorrhizal symbiosis: After 250 days, the biomass accumulation of *P. canadensis* was recorded (Table 1). The shoot and root biomass accumulation under different soil water regimes was significantly improved by the inoculation of *R. irregularis*, while drought stress limited plant growth. The root/shoot ratio was significantly affected by both *R. irregularis* and drought stress. No mycorrhizal symbiosis was observed in NM plant roots. Over 70% AM plant roots were colonized by *R. irregularis*, and the colonization was significantly reduced by drought stress.

Leaf RWC, SLA, leaf gas exchange parameters, and relative chlorophyll content: Compared with NM treatments, inoculation of *R. irregularis* increased leaf RWC and SLA under different water regimes, while drought stress decreased leaf RWC and SLA for both AM and NM treatments (Table 2).

Both inoculation of *R. irregularis* and drought stress significantly affected leaf gas exchange parameters and relative chlorophyll content (Fig. 1). With the intensified drought stress, P_N, g_s, C_i, *E*, *WUEi*, and relative chlorophyll content were decreased while the inoculation of *R. irregularis* increased these parameters compared with non-inoculated counterparts.

Xylem vulnerability curve and P_{50}: Vulnerability curves under different soil water regimes and mycorrhizal status were recorded (Fig. 2). All the vulnerability curves were "s" shaped. In the non-inoculated treatments, mild drought stress did not affect P_{50} while severe stress significantly decreased P_{50} (Table 2). Inoculation of *R. irregularis* did not affect P_{50} under well-watered condition, while decreased and maintained P_{50} under drought stress. Compared with NM treatment, inoculation of *R. irregularis* decreased P_{50} under mild stress and increased P_{50} under severe stress.

Correlation analysis: The colonization rate of *R. irregularis* was correlated with leaf RWC, P_N, g_s, and *WUEi* (Table 3). P_{50} was highly correlated with leaf RWC, SLA, P_N, g_s, C_i, *E*, and *WUEi*.

Table 1. Biomass and arbuscular mycorrhizal colonization rate of *Populus* × *canadensis*.

	Shoot FW (g)	Root FW (g)	Total FW (g)	Root/shoot ratio	Colonization rate (%)
WWAM	110.1 ± 7.6	89.3 ± 7.1a	199.4 ± 13.7a	0.81 ± 0.05	85.4 ± 2.0a
WWNM	94.7 ± 4.0	71.1 ± 2.1b	165.8 ± 4.8b	0.75 ± 0.04	0d
MSAM	95.1 ± 8.9	76.8 ± 5.3b	171.8 ± 6.1b	0.82 ± 0.13	77.2 ± 2.2b
MSNM	63.2 ± 10.2	39.2 ± 1.1c	102.5 ± 11.1c	0.63 ± 0.10	0d
SSAM	66.5 ± 6.5	35.4 ± 4.0c	102.0 ± 8.8c	0.53 ± 0.06	70.4 ± 0.6c
SSNM	46.7 ± 5.7	22.9 ± 3.7d	69.6 ± 5.5d	0.50 ± 0.11	0d
P_{WATER}	S	S	S	S	S
P_{AMF}	S	S	S	S	S
$P_{\text{WATER* AMF}}$	NS	S	S	NS	S

Note: WW, well-water; MS, mild stress; SS, severe stress; AM, inoculated with *Rhizophagus irregularis*; NM, non-mycorrhizal; S, significant; NS, not significant; Values with different letters indicated significant difference (LSD-test $P = 0.05$, n = 3)

Table 2. Leaf relative water content (RWC), specific leaf area (SLA), and xylem embolism vulnerability (P_{50}) of *Populus* × *Canadensis*.

	RWC	SLA (m^2 kg^{-1})	P_{50} (MPa)
WWAM	93.2 ± 1.7	121.3 ± 16.9	-1.99 ± -0.11a
WWNM	89.6 ± 0.8	96.3 ± 8.0	-2.26 ± -0.23ab
MSAM	87.6 ± 0.6	109.4 ± 6.2	-2.68 ± -0.16c
MSNM	84.8 ± 0.8	95.1 ± 3.6	-2.38 ± -0.20b
SSAM	81.9 ± 1.7	81.2 ± 7.3	-2.70 ± -0.12c
SSNM	78.8 ± 0.9	68.4 ± 20.4	-3.02 ± -0.14d
P_{WATER}	S	S	S
P_{AMF}	S	S	NS
$P_{\text{WATER* AMF}}$	NS	NS	S

Note: WW, well-water; MS, mild stress; SS, severe stress; AM, inoculated with *Rhizophagus irregularis*; NM, non-mycorrhizal; S, significant; NS, not significant; Values with different letters indicated significant difference (LSD-test $P = 0.05$, n = 3)

Table 3. Correlation coefficients among variables involves in RWC, SLA, leaf gas exchange, and xylem embolism vulnerability (P_{50}) of *Populus* × *canadensis*.

	RWC	SLA	P_N	g_s	C_i	*E*	*WUEi*
P_{50}	0.64**	0.84***	0.78***	0.77***	0.75***	0.81***	0.60**
Colonization	0.49*	0.40	0.55*	0.50*	0.46	0.39	0.61**

Note: RWC, relative water content; SLA, specific leaf area, P_N, net photosynthesis; C_i, intercellular CO_2 concentration; g_s, stomatal conductance, *E*, transpiration rate, *WUEi*, intrinsic water use efficiency; P_{50}, xylem embolism vulnerability.
*= Indicate significant different at $p<0.05$; **= Indicate significant different at $p<0.01$; ***= Indicate significant different at $p<0.001$

Discussion

Poplar is widely planted to meet various demands from wood production to ecological conservation, and requires a large amount of water and nutrient input (Eusemann *et al*., 2013; Zhou *et al*., 2020). *Populus* × *canadensis* 'Neva' is an important tree with many desirable characteristics (Liu *et al*., 2016). More than 70% of *P. canadensis* roots were colonized by *R. irregularis* in current study. This was observed in previous studies that *P. canadensis* could form mycorrhizal symbioses (Liu *et al*., 2016; Wu *et al*., 2017a, b). The established symbiosis increased plant biomass accumulation (Table 1), and was documented in several independent experiments (Liu *et al*., 2016; Wu *et al*., 2017a, b).

Root/shoot ratio is the index used to evaluate plant nutrient allocation (Hetrick, 1991). Although plant biomass accumulation was increased by *R. irregularis*, the root/shoot ratio was differently affected (Table 1). Plant root/shoot ratio was decreased by inoculation of AM fungi in previous studies using soybean (Wang *et al*., 2011), *Medicago truncatula* (Saravesi *et al*., 2014), and *Lycium barbarum* (Zhang *et al*., 2017). In addition, it was attributed to the reliance of plant on AM fungal improved nutrients and water exploration and uptake from soil (El-Mesbahi *et al*., 2012). However, in other studies using *Eucalyptus globulus* (Chen *et al*., 2000), *P. cathayana* (Chen *et al*., 2015), and *Robinia pseudoacacia* (Zhang *et al*., 2016b), the root/shoot ratio was not affected by AM fungi. The foraging strategies that different plants adopted for nutrient and water in soil may account for the different roles of AM fungi (Chen *et al*., 2016). The increased root/shoot ratio in current study might due to the clonally propagated *P. canadensis* which had specific forage strategy and nutrient allocation that would not be affected by *R. irregularis* (Veresoglou *et al*., 2012).

Better leaf RWC demonstrated that AM fungus inoculation improved poplar leaf water status under drought stress. Similar results were observed in previous plant-fungi interactions (Zhu *et al*., 2012; Ortiz *et al*., 2015) and it could be attributed to the AM fungi which improved water uptake and transportation through aquaporin genes expression regulation and hydraulic conductivity modulation (He *et al*., 2016; Sánchez-Romera *et al*., 2016; Hu *et al*., 2017).

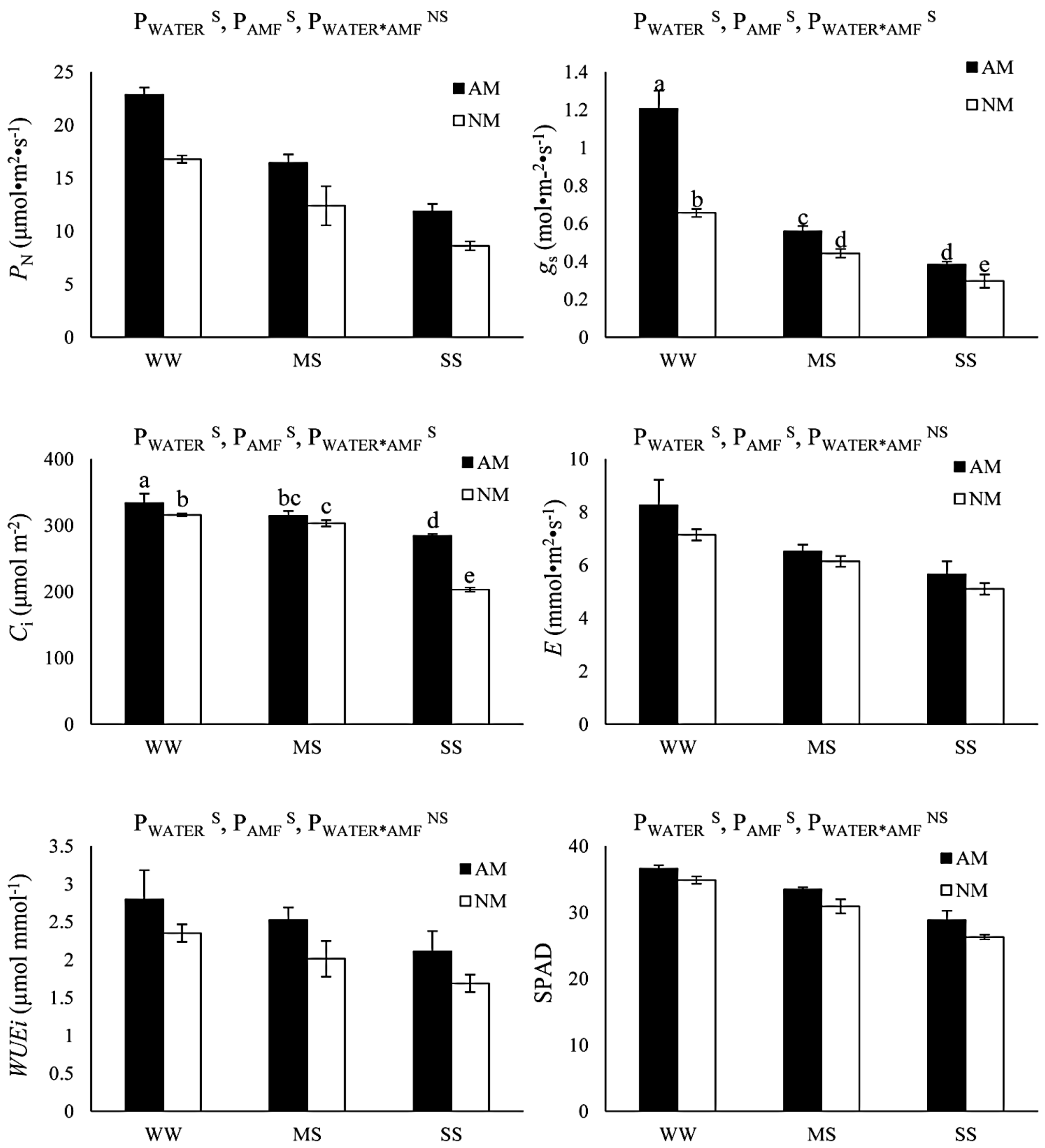

Fig. 1. Effect of AM fungus and water condition on gas exchange and chlorophyll related parameters. P_N, net photosynthesis; C_i, intercellular CO_2 concentration; g_s, stomatal conductance, *E*, transpiration rate, *WUEi*, intrinsic water use efficiency; WW, well-water; MS, mild stress; SS, severe stress; AM, inoculated with *Rhizophagus irregularis*; NM, non-mycorrhizal; s, significant; ns, not significant; Bars indicated means ± SD (n = 3). Values with different letters indicated significant difference (LSD-test *P* = 0.05, n = 3)

Under drought stress, SLA that indicate leaf area per leaf dry mass, was used to describe the variation of plant leaf structure (Marron *et al*., 2003) and plant growth potential (Poorter & Van der Werf, 1998). Reduced SLA demonstrated a higher mesophyll and protein density, and this was attributed to the drought affected leaf expansion (Liu & Stützel, 2004). For poplar, the reduction of SLA under drought stress was suggested genotype dependent (Monclus *et al*., 2006). In current study, drought stress showed a significant effect on SLA of *P. canadensis*. Reduced SLA could be considered as a limitation of biomass accumulation and photosynthesis, which were also observed in current study (Table 1 and Fig. 1). Positive influence of AM fungi on plant SLA was observed in previous studies (Zhang *et al*., 2016a; Sun *et al*., 2017), and was consistent with the inoculation of *R. irregularis* increased SLA under different water regimes in current study. The positive influence could attribute to the AM fungi improved plant water status discussed above and the improved N, P, and K nutrient (Zhang *et al*., 2016a; Wu *et al*., 2017; Zhang *et al*., 2017).

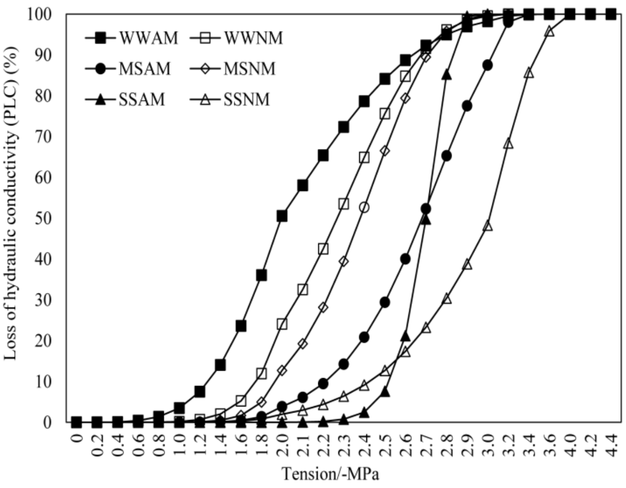

Fig. 2. Xylem vulnerability curve of *Populus × canadensis*under different treatments. WW, well-water; MS, mild stress; SS, severe stress; AM, colonized with *Rhizophagus irregularis*; NM, none-mycorrhizal.

AM fungi supply mineral nutrients and water in exchange of lipid from plant to complete life span (Jiang *et al*., 2017). Under drought stress, plants with AM symbiosis usually had higher biomass accumulation than those without the help of AM fungi (Sun *et al*., 2017; Zhang *et al*., 2017). In current study, the photosynthesis parameter was improved by the inoculation of *R. irregularis* (Fig. 1) and could explain the improved biomass accumulation. However, the reduction of drought stress on biomass accumulation was more obvious. As a woody species, hybrid poplar is generally considered to adopt relative isohydric behavior (Sade *et al*., 2012; Attia *et al*., 2015). The response of *P. canadensis* with AM symbiosis to drought stress in current study seemingly adopted anisohydric behavior. One possible explanation might be the AM fungus improved individual physiological condition via water and nutrient uptake. On the other hand, this might be that AM fungus regulated behavior change, as it demands photosynthates from host plants (Boldt *et al*., 2011; Augé *et al*., 2015).

As a tree species requires a large amount of water, poplar is considered drought sensitive and highly susceptible to xylem cavitation (Arango-Velez *et al*., 2011). In current study, the vulnerability curve of *P. canadensis* was "s" shaped (Fig. 2) and similar with other pure and clonal poplars analyzed in previous studies (Fichot *et al*., 2015). Under intensified water stress, the stem xylem vulnerability to cavitation was found to acclimate to the drought severity (Awad *et al*., 2010). The P_{50}, used in current study to represent xylem tension at which xylem hydraulic conductivity lost 50%, also decreased with the reduction of soil water content (Table 2). Inoculation of *R. irregularis* did not significantly increase P_{50} under well-watered condition, although the vulnerability curve in the inoculated treatment shifted toward higher xylem tension. Taken the stomatal conductance and transpiration rate increment into consideration, inoculation of *R. irregularis* under well-watered condition maintained the resistance of *P. canadensis* to xylem cavitation as the driving force for water uptake increased (Tyree & Sperry, 2003; Meinzer & McCulloh, 2013). Under drought stressed conditions, the poplar inoculated with *R. irregularis* maintained P_{50} despite drought severity. Moreover, this might be considered as the decreased drought sensitivity of *P. canadensis* by inoculation of *R. irregularis*. A possible explanation might be the 'priming' effect of AM fungi against drought stress as they used for biotic stress (Jung *et al*., 2012). To guarantee photosynthetic gas change and growth, the water flow from root to leaf must be assured and the xylem cavitation should be eliminated (Attia *et al*., 2015). In current study, the P_{50} was highly correlated with leaf RWC, SLA, and photosynthetic parameters (Table 3). This indicated a linkage between water consumption and maintenance of water transport (Klein, 2014). Colonization rate of *R. irregularis* showed correlation with leaf RWC and some photosynthetic parameters but the correlation coefficients were smaller than that of P_{50}. This indicated an indirect link between colonization status and influence of plant physiology under drought stress (Marulanda *et al*., 2006).

Conclusion

Our results indicated that inoculation of *R. irregularis* improved biomass accumulation, leaf RWC, SLA, and photosynthesis of *P. canadensis* under different water regimes. The xylem vulnerability to cavitation of *P. canadensis* was acclimated to drought stress, and modulated by the inoculation of *R. irregularis* under intensified drought stress. However, the specific influence of AM fungus on the plant traits that affect xylem vulnerability to cavitation remains elusive. Since AM fungi could systematically regulate plant genes expression (Calabrese *et al*., 2017), further studies focus on the regulation of AM fungi on plant genes involved in xylem cavitation development could unveil the role of AM fungi in strengthening plant resistance of xylem cavitation and drought tolerance.

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