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ORIGINAL ARTICLE

Arbuscular mycorrhizal fungal colonization improves growth, photosynthesis, and ROS regulation of split-root poplar under drought stress

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Abstract

Arbuscular mycorrhizal (AM) fungi form ubiquitous symbioses with terrestrial plants in different ecosystems and provide a variety of benefits including improved drought tolerance of host plants. However, the difference and contribution of colonized and un-colonized root-system parts within mycorrhizal plants against drought stress is uncertain. A split-root system was used and the root compartments were either non-inoculated or inoculated with *Rhizophagus irregularis*, and were subjected to either well-watered or drought-stressed conditions. The growth, photosynthesis, reactive oxygen species (ROS) scavenging, and relative gene expression of aquaporins and phosphate transporters of hybrid poplar (*Populus* × *canadensis* 'Neva') were evaluated. Our results indicated that the inoculation by *R. irregularis* in either one or both compartments of split-root systems increased poplar biomass accumulation, photosynthesis, and ROS regulation under well-watered and drought-stressed conditions. When inoculum was applied in both compartments of split-root systems, the beneficial effect of *R. irregularis* was greater than that in treatment where only one compartment received inoculum. The effect of *R. irregularis* may attribute to improved phosphorus uptake via upregulation of relative expressions of *PcPT3*, *PcPT4*, *PcPT5*, and a possible improvement of water uptake via modulation of aquaporins (*PcPIP2-3*, *PcPIP2-5*, *PcTIP1-1*, and *PcTIP1-2*) in colonized root-system parts. Our results demonstrated that the benefits of the AM symbiosis depend on the extent of root colonization through which AM fungus may modulate plant phosphate and water uptake to improve tolerance of poplar against drought stress.

Keywords Arbuscular mycorrhizal fungus · Aquaporins · Poplar · Phosphate transporters · Photosynthesis · Reactive oxygen species

Introduction

Drought stress occurs frequently in northwest areas of China, and limits plant growth and productivity (Yan et al. 2017). In response to drought stress, plants induce several physiological and biochemical changes (Liu et al. 2016). One physiological change is the reduction of photosynthesis and stomatal conductance to avoid water loss through leaves (Lawlor and Cornic 2002). Another biochemical change is the build-up of reactive oxygen species (ROS) that due to electron leakage in chloroplast and mitochondria and NADPH oxidization (Ahmed et al. 2009; Gill and Tuteja 2010; Marino et al. 2012). Plants use antioxidative enzymes and small molecular compounds to scavenge ROS (Gill and Tuteja 2010).

Arbuscular mycorrhizal (AM) fungi are widely distributed in different ecosystems and form symbioses with most terrestrial plants (Smith and Read 2008). Plants that form

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AM symbioses acquire numerus benefits, including nutrient and water uptake through additive mycorrhizal pathway from soil (Smith et al. 2011; Liu et al. 2019b; Wang et al. 2020). In exchange for the benefits, the AM fungi receive photosynthates from host plants (Pfeffer et al. 1999; Jiang et al. 2017), and host plants balance the cost–benefit of the symbiosis by controlling the delivery photosynthates to the AM fungi (Vierheilig 2004; Meixner et al. 2005). Moreover, the AM symbiosis improves plants that under drought stress photosynthesis and stomatal conductance, which has been attributed to the improved water uptake by AM fungi (Liu et al. 2015; Li et al. 2015; He et al. 2017).

Aquaporins form water membrane channels for passive water movement in plants (Maurel et al. 2008). Aquaporins control transcellular water transport and the hydraulic conductance in plants, and limit symplastic water transport to prevent water loss under drought stress. In accompany with the regulation of aquaporin genes expression (He et al. 2016; Quiroga et al. 2017), AM symbiosis improves hydraulic conductance and water status of host plants (Calvo-Polanco et al. 2016; Kilpeläinen et al. 2020). Moreover, aquaporin-mediated long-distance phosphate transport in AM fungal hyphae (Kikuchi et al. 2016) may assist phosphate transport from AM fungi to the plant in conjunction with the phosphate transporters from the PHT1 family (Nussaume et al. 2011; Smith et al. 2011). Under drought stress, mycorrhizal plants additionally have higher activities of antioxidative enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), and higher contents of glutathione (GSH) than the non-mycorrhizal plants. These changes have been attributed to the regulation of genes for signal transduction and enzyme synthesis associated with ROS scavenging systems (He et al. 2017, 2020; Huang et al. 2020), that improve scavenging of ROS in mycorrhizal plants and reduce the content of H_2O_2 and malondialdehyde (MDA), which are products of lipid oxidation (Wu et al. 2006; Liu et al. 2016; He et al. 2017).

Populus spp. are fast-growing woody plants, and have great value for paper industry, biomass production, and ecological conservation (Luo and Polle 2009; Hacke et al. 2010). *Populus* × *canadensis* (a hybrid of *P. nigra* × *P. deltoides*) 'Neva' is widely planted in semi-arid areas of northwest China, and its growth is limited by drought stress. In our previous studies, photosynthesis, antioxidative responses, and aquaporin genes expression of this poplar hybrid under drought stress were improved by inoculation with AM fungi (Liu et al. 2015, 2016). Further, the effect of AM symbiosis on this poplar hybrid was promoted when the colonization rate of root increased (Wu et al. 2017). The results raised a question of whether the improvement of AM symbiosis on this poplar hybrid was depended on only the portion of the AM fungal-colonized root-system parts, or was systemically regulated host physiological responses, or both. To answer this question, a split-root system was used to evaluate the effects of AM fungal colonization and poplar response to drought. The root compartments of the split-root system were either non-inoculated or inoculated with *Rhizophagus irregularis*, and were subjected to either well-watered or drought-stressed conditions. Plants growth, photosynthesis, ROS scavenging, and relative expression of aquaporin and phosphate transporter genes of this hybrid poplar were evaluated.

Materials and methods

Plant material, growth substrate, and AM fungal inoculum

Hybrid poplar (*P.* × *canadensis* 'Neva') cuttings were collected and disinfected as described by Li et al. (2021). The cuttings were grown in plastic cups (5 cm in diameter and 7.5 cm in height) containing 0.2 kg growth substrate (without rooting hormones) for 4 months. When there were 6 leaves, the cuttings were used for split-root experiment. The cuttings were watered every other day and fertilized with 10 mL full-strength Hoagland's solution every 2 weeks (Hoagland and Arnon 1938).

Growth substrate and the Inoculum of *R. irregularis* were prepared as described by Li et al. (2021).

Experimental design and growth condition

Poplar cuttings were cultivated in split-root systems (Fig. 1) that were made of acrylic plates. Growth substrate (800 g) was added in each root compartment (7 cm × 7 cm × 12 cm, length × width × height). The split-root system was used to evaluate the influence of two factors: (1) AM status, inoculated with *R. irregularis* inoculum (A) or inoculated with autoclaved inoculum (N); (2) water status, well-watered (W, 70–75% of field capacity) or drought stressed (D, 30–35% of field capacity). When poplar cuttings were transplanted into the split-root systems, 10 g inoculum was applied underneath roots for mycorrhizal treatments while the non-mycorrhizal treatments received autoclaved inoculum with inoculum filtrate (He et al. 2016). The poplar cuttings were divided into 3 groups: (1) cuttings with both root system halves inoculated; (2) cuttings with both root system halves non-inoculated; (3) cuttings with only one root system half inoculated. Four weeks post transplanting, water treatment started and lasted for 8 weeks, where half of the split-root system with different AM status groups were irrigated with limited water amount. The TDR100 system (Campell) was used (once every other day) to monitor soil moisture, of which the 100% soil water-holding capacity of growth

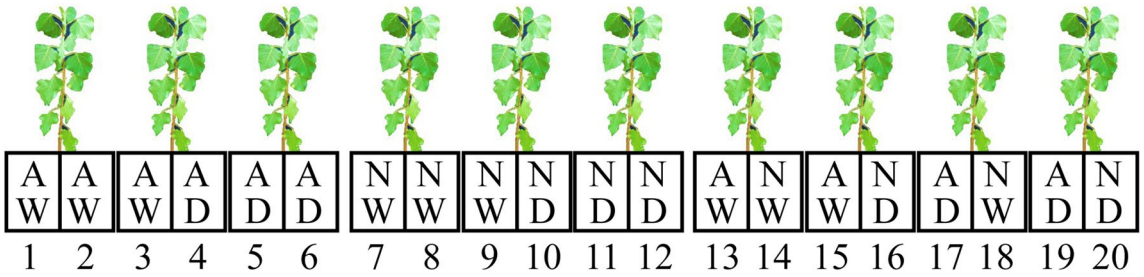

Fig. 1 Split-root system established for studies of interaction of *R. irregularis* and drought stress on poplar. The letter A in root compartment indicates inoculation of *R. irregularis* and N indicates mock inoculation. The letter W in root compartment indicates well-watered condition (70–75% of field capacity) and D indicates drought-stressed condition (30–35% of field capacity). The shoot treatments were named as the combination of 4 letters in root compartments, and the root system halves were named as the combination of number and letters in root compartments

substrate corresponds to 20% volumetric soil moisture. At harvest (12 weeks after transplantation), 10 treatments were as follow: (1) AW/AW; (2) AW/AD; (3) AD/AD; (4) NW/NW; (5) NW/ND; (6) ND/ND; (7) AW/NW; (8) AW/ND; (9) AD/NW; (10) AD/ND. One poplar cutting was grown in each split-root system and each treatment had four replicates.

The split-root systems were arranged in a randomized complete block design and placed in greenhouse as described by Li et al. (2021). Throughout the experiment, the poplar cuttings were irrigated with water to maintain the two treatments, and fertilized by applying 20 mL of full-strength Hoagland's solution in each root compartment once every 2 weeks.

Biomass accumulation and AM fungal colonization

Harvest was conducted 12 weeks post transplantation, and the shoot and root were separated. Roots from each root compartment were separately collected, thoroughly washed, and dried with paper towels. Leaves from each shoot treatment were evenly divided into two parts: one part was used for enzymatic parameter measurement; one part was used for fresh weight and dry weight ratio calculation. Roots from each compartment were divided into three parts: one part was used for observation of AM fungal colonization; one part was used for assessment of enzymatic activity and gene expression; one part was used for fresh weight and dry weight ratio calculation. Dry weight (DW) of branches, leaves, and root was measured as described by Li et al. (2021) for four cuttings per treatment combination ($n=4$).

The observation and measurement of AM fungal colonization used part of fresh root from each root compartment ($n=4$). The root was stained (Hu et al. 2016), and over 200 intersections of over 20 stained root segments (5 cm long) from each compartment were observed with × 400 magnification under a light microscope and the gridline intersect method was applied (Giovannetti and Mosse 1980).

Gas exchange measurement and leaf relative water content

One day before harvest, the gas exchange parameters were measured as described by Li et al. (2021). The leaf relative water content was measured as described by Liu et al. (2015). The analysis was done in four independent cuttings per treatment.

Antioxidation enzymes activity, soluble protein concentration, H_2O_2 concentration, malondialdehyde (MDA) concentration, and phosphorus concentration

Fully expanded, healthy leaves and roots were collected, homogenized in liquid nitrogen, and 0.5 g leaves and roots samples were used to analyze antioxidation enzyme activity, soluble protein concentration, H_2O_2 concentration, and MDA concentration as described by He et al. (2017). The phosphorus concentration was determined as described by Li et al. (2021). The analysis was done in four independent leaves and root samples per treatment.

RNA extraction and first-strand cDNA synthesis

RNA extraction, RNA quality and quantity assessment, and first-strand cDNA synthesis was conducted as described by Liu et al. (2016).

Quantitative real-time PCR (qRT-PCR) analysis

Full-length cDNA sequences encoding the phosphate transporters in the PHT1 family and aquaporins were obtained from transcriptomic analysis, designated *PcPT3*, *PcPT4*, *PcPT5*, *PcPT9*, *PcPIP1-1*, *PcPIP1-2*, *PcPIP1-3*, *PcPIP2-1*,

PcPIP2-2, *PcPIP2-3*, *PcPIP2-4*, *PcPIP2-5*, *PcTIP1-1*, and *PcTIP1-2* (supplementary Figure S1 and S2), and deposited in GenBank (MN546004-MN546007, MN546012-MN546014, MN546016–MN546020, MN546022, and MN546023).

qRT-PCR was performed to analyze the transcript accumulation of genes encoding phosphate transporters and aquaporins of roots from each compartment (20 compartments in total) of the split-root system. Primers used for qRT-PCR were listed in Table S1. To ascertain the uniqueness of the product of primers, the products were transformed into 18-T vector and sequenced. qRT-PCR amplifications were performed as described by Liu et al. (2016). All qRT-PCR reactions were performed with 4 biological replications (from each root system half) and 3 technical repetitions. A unique fragment of 18S rRNA gene (guanine-*N*(7)-methyltransferase) of *P.* × *canadensis* 'Neva' was used for normalization. The relative expression were calculated as $2^{-\Delta CT}$ ($\Delta CT = CT^{\text{gene of interest}}$ minus $CT^{18S\ rRNA}$) (Zhang and Franken 2014).

Statistical analysis

Statistical analysis was performed using the SPSS 20.0 statistical program (SPSS Inc., Chicago, IL, USA). MANOVA was performed separately to detect the main contributions of *R. irregularis* and drought stress to the different parameters studied for leaves and root system halves. When MANOVA indicated a significant effect (Wilk's lambda, $P < 0.05$), the individual effects of the presence of *R. irregularis* and drought stress in none, one, or both root compartments were tested. When MANOVA indicated a significant interaction between AM fungus and drought stress, one-way ANOVA and post hoc Tukey's HSD tests were performed. For root colonization rate, one-way ANOVA and post hoc Tukey's HSD test were performed. The Shapiro–Wilk's test was used to test the assumptions of normality, and the Levene's test was used for the test of equal variance. The heatmap and hierarchical clustering analysis was performed using the MetaboAnalyst 4.0 (Chong et al. 2018).

Results

Plant biomass accumulation and mycorrhizal colonization rate

Drought stress reduced the dry weight (DW) of shoots and roots, and the reduction was intensified when both of the root compartments were subject to the drought-stressed

Table 1 Effect of drought stress and inoculation of *Rhizophagus irregularis* in the split-root system on growth for shoot, parameters of gas exchange, relative water content, enzymatic responses, and oxidative damage of leaves in poplar

	Drought treatment			*R. irregularis* treatment		
	Two sides drought	One side drought	No drought	No *R. irregularis*	One side inoculated	Two sides inoculated
Shoot DW (g)	2.21 ± 0.18 C	3.05 ± 0.22 B	3.93 ± 0.40 A	2.27 ± 0.19 C	2.87 ± 0.17 B	4.11 ± 0.39 A
P concentration (g/kg)	0.729 ± 0.031 A	0.728 ± 0.042 A	0.761 ± 0.041 A	0.719 ± 0.021 B	0.741 ± 0.019 B	0.782 ± 0.009 A
RWC (%)	66.8 ± 1.4 B	70.4 ± 1.1 B	76.8 ± 1.1 A	68.8 ± 1.8 B	71.0 ± 1.6 AB	74.0 ± 1.4 A
Pn (μmol CO_2/m^2 s)	12.7 ± 0.8 C	17.4 ± 0.5 B	19.9 ± 0.5 A	15.4 ± 1.5 B	16.2 ± 0.7 B	18.9 ± 1.0 A
g_s (mol H_2O/m^2 s)	0.331 ± 0.022 C	0.404 ± 0.024 B	0.541 ± 0.012 A	0.406 ± 0.028 B	0.390 ± 0.037 B	0.480 ± 0.021 A
C_i (μmol CO_2/m^2 s)	225 ± 4 C	292 ± 5 B	316 ± 7 A	298 ± 17 A	271 ± 11 B	270 ± 11 B
E (mmol H_2O/m^2 s)	2.33 ± 0.12 C	4.54 ± 0.19 B	5.89 ± 0.21 A	3.72 ± 0.46 B	4.44 ± 0.45 A	4.62 ± 0.53 A
WUEi (μmol/mol)	5.46 ± 0.19 A	3.88 ± 0.12 B	3.41 ± 0.14 C	4.3 ± 0.20 A	4.02 ± 0.34 A	4.38 ± 0.33 A
APX (mmol/gFW min)	2.19 ± 0.30 A	1.93 ± 0.20 A	1.13 ± 0.11 B	0.94 ± 0.07 C	2.31 ± 0.22 A	1.87 ± 0.14 B
CAT (U/gFW min)	216 ± 10 C	303 ± 18 B	352 ± 23 A	226 ± 15 C	298 ± 16 B	349 ± 28 A
H_2O_2 (μmol/g)	3.32 ± 0.26 A	2.77 ± 0.08 B	2.44 ± 0.08 C	3.3 ± 0.26 A	2.82 ± 0.07 B	2.39 ± 0.07 C
POD (μg/g min)	34.3 ± 3.2 C	47.1 ± 2.2 B	63.1 ± 5.2 A	38.1 ± 4.0 C	45.6 ± 2.6 B	61.3 ± 5.8 A
Soluble Protein (mg/g)	2.45 ± 0.32 C	3.42 ± 0.25 B	5.34 ± 0.34 A	2.83 ± 0.50 B	3.99 ± 0.19 A	4.21 ± 0.65 A
MDA (μmol/gFW)	19.1 ± 2.4 A	12.5 ± 0.4 B	10.1 ± 0.4 C	18.1 ± 2.7 A	11.9 ± 0.7 B	12.0 ± 0.7 B
GSH (μmol/g)	13.1 ± 0.4 A	11.7 ± 0.4 B	11.2 ± 0.4 B	12.4 ± 0.6 A	12.1 ± 0.4 A	11.3 ± 0.3 A
SOD (U/gFW min)	83.2 ± 5.6 A	69.9 ± 4.0 B	59.0 ± 5.6 C	92.3 ± 3.6 A	64.9 ± 2.9 B	56.7 ± 3.5 C

Data were means (± SE), $n = 4$; different letters within the same line indicate significant differences as determined using MANOVA ($\alpha = 0.05$) for the effect of either drought treatment or *R. irregularis* treatment

DW dry weight, *RWC* relative water content, P_N net photosynthetic rate, g_s stomatal conductance, C_i intercellular CO_2 concentration, *E* transpiration rate, *WUEi* intrinsic water use efficiency, *SOD* superoxide dismutase, *POD* peroxidase, *CAT* catalase, *APX* ascorbate peroxidase, *GSH* glutathione, *MDA* malondialdehyde

Table 2 Effect of drought stress and inoculation of *Rhizophagus irregularis* in the split-root system on root growth, colonization, parameters of enzymatic response and oxidative damage, and relative gene expression of phosphate transporters and aquaporins of poplar

	Drought treatment			*R. irregularis* treatment		
	Two sides drought	One side drought	No drought	No *R. irregularis*	One side inoculated	Two sides inoculated
Root DW (g)	0.883 ± 0.061 C	1.24 ± 0.09 B	1.49 ± 0.07 A	0.985 ± 0.055 B	1.29 ± 0.08 A	1.32 ± 0.11 A
P concentration (g/kg)	0.821 ± 0.007 B	0.881 ± 0.009 A	0.887 ± 0.011 A	0.855 ± 0.016 A	0.862 ± 0.018 A	0.865 ± 0.008 A
APX (mmol/gFW min)	1.77 ± 0.12 A	1.39 ± 0.13 B	0.91 ± 0.06 C	1.00 ± 0.09 C	1.36 ± 0.12 B	1.73 ± 0.15 A
CAT (U/gFW min)	192 ± 4 A	184 ± 3 A	181 ± 5 A	196 ± 3 A	185 ± 3 AB	175 ± 4 B
H_2O_2 (μmol/g)	0.198 ± 0.004 A	0.126 ± 0.001 B	0.104 ± 0.005 C	0.138 ± 0.011 A	0.139 ± 0.011 A	0.147 ± 0.008 A
POD (μg/g min)	77.2 ± 5.8 A	54.9 ± 5.8 B	30.4 ± 1.9 C	68.2 ± 7.4 A	51.4 ± 5.6 B	44.0 ± 5.7 B
Soluble Protein(mg/g)	2.07 ± 0.24 C	3.10 ± 0.29 B	3.84 ± 0.36 A	1.77 ± 0.13 C	3.04 ± 0.32 B	4.22 ± 0.22 A
MDA (μmol/gFW)	2.80 ± 0.10 A	2.24 ± 0.09 B	1.75 ± 0.04 C	2.36 ± 0.14 A	2.31 ± 0.13 A	2.11 ± 0.10 A
GSH (μmol/g)	2.68 ± 0.08 A	2.27 ± 0.09 B	1.96 ± 0.06 C	2.44 ± 0.09 A	2.24 ± 0.09 A	2.25 ± 0.13 A
SOD (U/gFW min)	46.6 ± 1.2 A	35.6 ± 1.9 B	30.5 ± 0.8 B	40.1 ± 2.0 A	34.5 ± 1.9 B	38.6 ± 2.3 AB
PcPT3 (M)	3.92 ± 0.74 A	3.44 ± 0.65 A	3.79 ± 0.76 A	0.121 ± 0.724 C	3.87 ± 0.69 B	7.00 ± 0.72 A
PcPT4 (M)	1.27 ± 0.16 A	1.20 ± 0.16 A	0.943 ± 0.176 A	0.491 ± 0.137 B	1.13 ± 0.10 B	1.82 ± 0.15 A
PcPT5 (M)	0.755 ± 0.169 B	0.929 ± 0.186 B	1.18 ± 0.22 A	0.087 ± 0.150 C	0.986 ± 0.180 B	1.77 ± 0.20 A
PcPT9 (M)	− 0.602 ± 0.116 B	− 0.365 ± 0.115 B	− 0.142 ± 0.099 A	− 0.137 ± 0.103 A	− 0.276 ± 0.088 B	− 0.726 ± 0.106 B
PcPIP1-1 (M)	− 0.997 ± 0.231 B	− 0.399 ± 0.197 B	− 0.267 ± 0.104 A	− 0.250 ± 0.098 A	− 0.365 ± 0.104 B	− 1.06 ± 0.13 B
PcPIP1-3 (M)	− 0.193 ± 0.347 A	− 0.103 ± 0.256 A	0.772 ± 0.299 A	0.221 ± 0.142 A	0.450 ± 0.179 A	− 0.380 ± 0.376 A
PcPIP2-1 (M)	− 0.565 ± 0.232 B	− 0.166 ± 0.130 B	0.363 ± 0.118 A	− 0.305 ± 0.133 A	− 0.129 ± 0.208 A	0.0540 ± 0.196 A
PcPIP2-2 (M)	− 0.688 ± 0.141 B	− 0.199 ± 0.180 B	0.448 ± 0.169 A	− 0.378 ± 0.220 A	− 0.159 ± 0.153 A	0.0845 ± 0.213 A
PcPIP2-3 (M)	1.25 ± 0.34 A	1.22 ± 0.252 A	1.26 ± 0.28 A	− 0.252 ± 0.271 C	1.42 ± 0.27 B	2.49 ± 0.34 A
PcPIP2-4 (M)	− 1.48 ± 0.17 B	− 0.874 ± 0.173 B	0.0707 ± 0.118 A	− 0.462 ± 0.165 A	− 0.788 ± 0.179 A	− 1.07 ± 0.13 A
PcPIP2-5 (M)	0.494 ± 0.189 A	0.700 ± 0.146 A	0.816 ± 0.184 A	0.051 ± 0.181 C	0.734 ± 0.137 B	1.21 ± 0.16 A
PcTIP1-1 (M)	0.636 ± 0.122 A	0.728 ± 0.106 A	0.848 ± 0.160 A	0.213 ± 0.125 B	0.769 ± 0.124 B	1.22 ± 0.14 A
PcTIP1-2 (M)	1.277 ± 0.155 A	1.195 ± 0.175 A	0.943 ± 0.176 A	0.491 ± 0.137 B	1.13 ± 0.10 B	1.82 ± 0.15 A

The relative gene expression was shown as the M ratio ($M = Log_2^{treatment/control}$, control was root without neither drought treatment nor *R. irregularis* treatment); data were means (± SE), $n = 4$; different letters within the same line indicate significant differences as determined using MANOVA ($\alpha = 0.05$) for the effect of either drought treatment or *R. ir DW* dry weight, *SOD* superoxide dismutase, *POD* peroxidase, *CAT* catalase, *APX* ascorbate peroxidase, *GSH* glutathione, *MDA* malondialdehyde

regularis treatment

condition (Tables 1, 2, and Supplementary Table S2, S5). Inoculation by *R. irregularis* in either one or both root compartments increased the DW of shoot and root. The increment of shoot DW in treatment with *R. irregularis* in both root compartments was higher than that in treatment with *R. irregularis* in only one root compartment (Fig. 2).

Mycorrhizal colonization was only observed in root compartments that received *R. irregularis* inoculum (Fig. 2b). The colonization rate was greater in plants growing with only one compartment inoculated than in plants with both compartments inoculated. Drought stress limited the colonization rate only when drought stress was applied in root compartments that received inoculum.

Gas exchange, RWC, phosphorus concentration, enzymatic response, and oxidative damage of poplar leaves

Drought stress reduced net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), intercellular CO_2 concentration (C_i), and RWC of poplar leaves (Supplementary Table S2). These reductions occurred when one of the compartments was subjected to drought stress, and intensified when both of the compartments were subjected to drought stress (Table 1). In contrast, the water use efficiency (WUEi) increased with the increment of compartments subjected to drought stress. The presence of *R.*

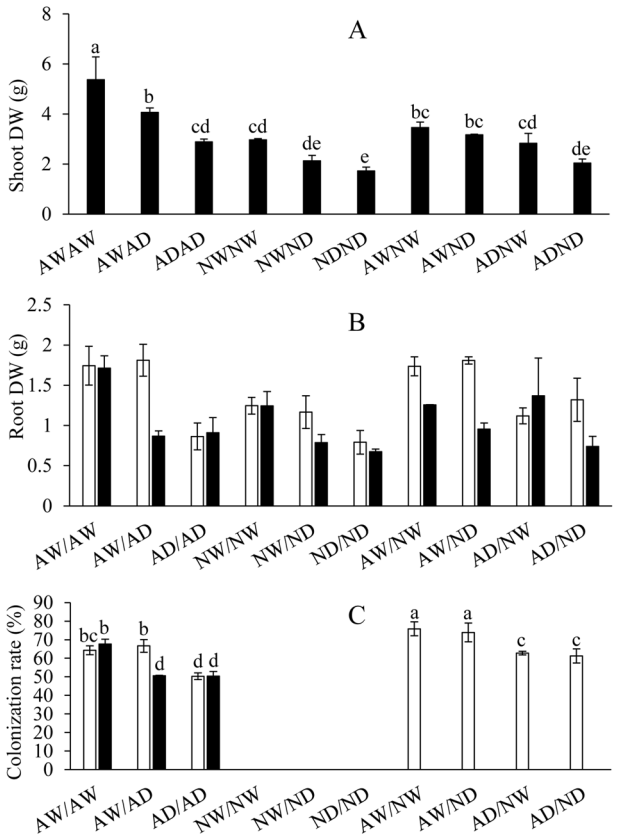

Fig. 2 The shoot dry weight and colonization rate of roots in split-root system. The root compartments of the split-root system were either non-inoculated (N) or inoculated with *Rhizophagus irregularis* (A), and were subjected to either well-watered (W) or drought stressed (D). Data were shown as mean ± SD. The white bars represent the root system halves on the left part of plant root in Fig. 1, and the black represent the root system halves on the right part of plant root in Fig. 1. Means followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's HSD-tests, $n = 4$)

irregularis in both compartments of the split-root system resulted in the increase of P_N, g_s, E, and RWC. The presence of *R. irregularis* in either one or both compartments only reduced the C_i, but not the *WUEi* (Table 1, Supplementary Tables S2, S4).

The phosphorus concentration of poplar leaves was increased when both compartments received inoculum, but not affected by drought-stress (Supplementary Table S2, Table 1).

Drought stress in either one or both compartments increased the activity of SOD and APX, increased the concentrations of H_2O_2, MDA, and GSH, and decreased the activity of POD and CAT and the soluble protein concentration (Table 1, Supplementary Tables S2, S5). The presence of *R. irregularis* in either one or two compartments increased the activity of APX, CAT, and POD, increased the concentration of soluble protein, and decreased the activity of SOD and the concentration of H_2O_2 and MDA.

Relative expression of genes, phosphorus concentration, and enzymatic response and oxidative damage in poplar roots from each compartment

The expression of genes encoding phosphate transporters and aquaporins of roots from each compartments of the split-root system were assessed and normalized by the expression of a unique plant 18S rRNA gene (Fig. 3). The relative expression of *PcPT3*, *PcPT4*, and *PcPT5* were upregulated by fungal inoculation, but not affected by drought stress (Supplementary Table S5). The upregulation of these genes occurred when *R. irregularis* was inoculated in either one or both of the compartments (Table 2). The relative expression of *PcPT9* was downregulated by both fungal inoculation and drought stress. The downregulation occurred when *R. irregularis* inoculum or drought stress was applied in both compartments. The phosphorus concentration of poplar roots was reduced when both compartments were under drought stress, but not affected by the fungal inoculation (Supplementary Table S5, Table 2).

When drought stress was applied in both of the root compartments, the relative expression of *PcPIP1-1*, *PcPIP2-1*, *PcPIP2-2*, *PcPIP2-4*, and *PcTIP1-2* was downregulated (Table 2, Supplementary Table S5). *PcPIP2-2*, *PcPIP2-4*, and *PcTIP1-2* were also downregulated when only one compartment of the split-root system was subjected to drought stress (Table 2). The presence of *R. irregularis* in both of the root compartments upregulated the relative expression of *PcPIP2-3*, *PcPIP2-5*, *PcTIP1-1*, and *PcTIP1-2,* and downregulated the relative expression of *PcPIP1-1* (Table 2). *PcPIP2-3* and *PcPIP2-5* were also upregulated when *R. irregularis* inoculum was applied in only one compartment (Table 2, Supplementary Table S6).

The enzymatic response and oxidative damage of root systems were assessed (Fig. 4, Table S7). Drought stress in either one or both compartments increased the activity of APX and POD, increased the concentration of GSH, H_2O_2, and MDA, and decreased the concentration of soluble protein (Supplementary Table S5, Table 2). When both root compartments were subjected to drought stress, the activity of SOD increased. The presence of *R. irregularis* in either one or both root compartments increased the activity of APX and the concentration of soluble protein, and decreased the activity of POD. When *R. irregularis* was present in both compartments, the activity of CAT declined (Supplementary Table S5, Table 2). Drought stress and AM symbiosis showed a significant interaction ($P < 0.001$) for H_2O_2 concentration, indicating an involvement of *R. irregularis* in modulating the H_2O_2 concentrations in roots.

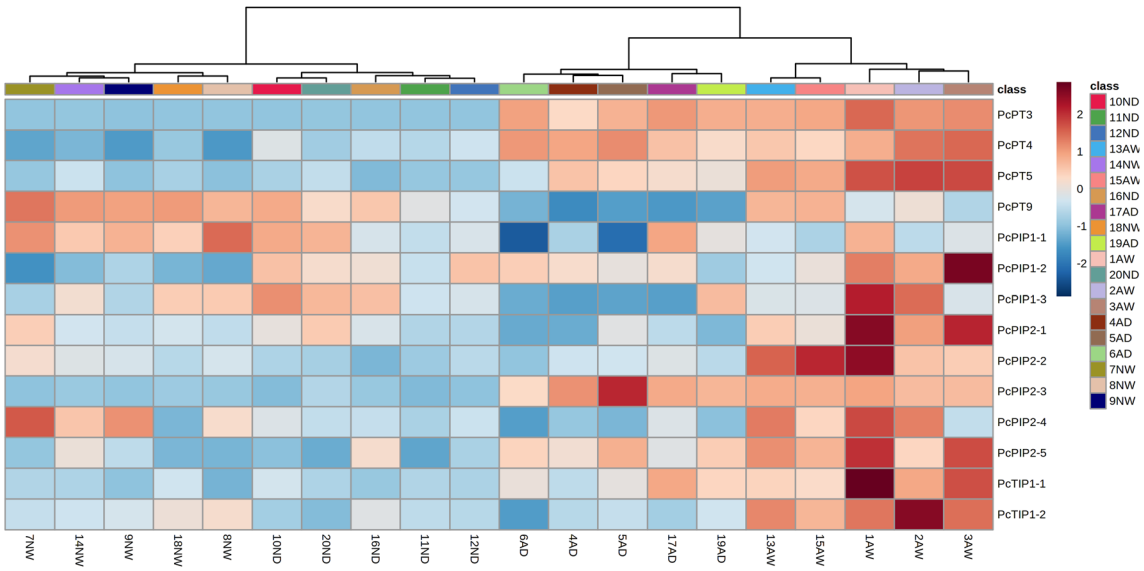

Fig. 3 The relative expressions of genes of PHT1 family and aquaporins in poplar roots. The root compartments of the split-root system were either non-inoculated (N) or inoculated with *Rhizophagus irregularis* (A), and were subjected to either well-watered (W) or drought stressed (D). The analysis used data including relative expression of *PcPT3*, *PcPT4*, *PcPT5*, *PcPT9*, *PcPIP1-1*, *PcPIP1-2*, *PcPIP1-3*, *PcPIP2-1*, *PcPIP2-2*, *PcPIP2-3*, *PcPIP2-4*, *PcPIP2-5*, *PcTIP1-1*, and *PcTIP1-2* in poplar roots. The genes expression was normalized by the expression of a unique fragment of plant 18S rRNA gene

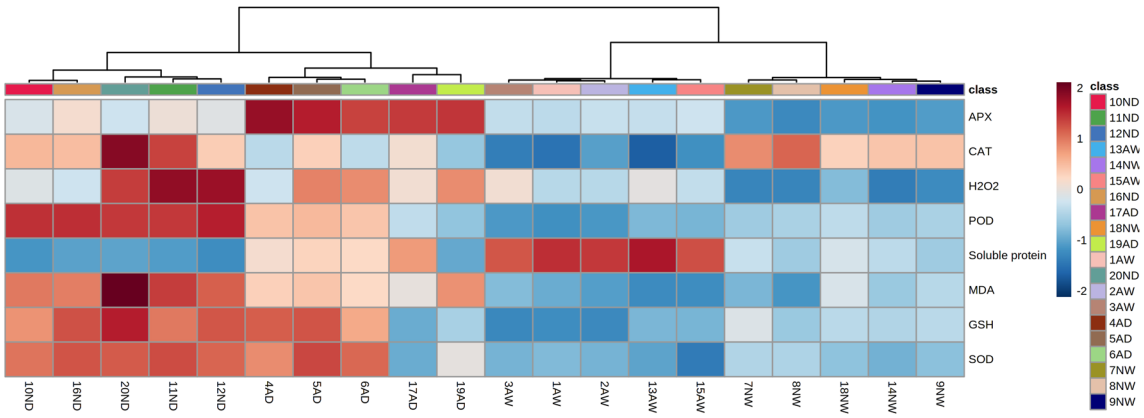

Fig. 4 The activity of enzymes, the concentration of small molecular compounds, and oxidative damage in poplar roots. The root compartments of the split-root system were either non-inoculated (N) or inoculated with *Rhizophagus irregularis* (A), and were subjected to either well-watered (W) or drought stressed (D). The analysis used data including activity of SOD, POD, CAT, and APX, content of H_2O_2, glutathione, and MDA in poplar roots

Discussion

Poplars serve economic and ecological purposes, and are widely planted in areas of northwest China where water deficiency is common (Hacke et al. 2010; Yan et al. 2017). In this study, over 50% roots in root compartments that received inoculum were colonized by *R. irregularis* (Fig. 2). This was similar with other studies of poplars and AM fungi (Cicatelli et al. 2010; Wu et al. 2017; De Oliveira et al. 2020). To control the loss of photosynthates that AM fungi demand, plants may maintain the colonization rate of the whole root system at a certain level (Vierheilig 2004; Meixner et al. 2005), and this might result in the higher colonization rate of poplar roots when only one root compartment received inoculum (Fig. 2) (Wang et al. 2018). Drought stress limits both photosynthesis of plant and development of AM fungal hyphae in soil, and consequently reduces colonization rate (Neumann et al. 2009; Zhang et al. 2018; Leyva-Morales et al. 2019). Another

possible reason may be the biosynthesis of strigolactones that differently respond to drought stress and AM symbiosis, and modulates plants interact with AM fungi (Ruiz-Lozano et al. 2016; López-Ráez 2016).

Biomass accumulation is the most distinct index that shows the response of plant to drought stress. The presence of *R. irregularis* in one root compartment increased the poplar shoot DW that were independent of drought stress, and the effect of *R. irregularis* was further promoted when both root compartments were inoculated (Table 1). This result is similar with other studies using split-root systems (Neumann et al. 2009; Li et al. 2016), and might be due to the thoroughly exploited water and mineral nutrients in growth substrate by AM fungal hyphae (Bitterlich et al. 2018), which have higher nutrient and water foraging capability than roots (Smith and Read 2008; Liu et al. 2019a). Moreover, the presence of *R. irregularis* in either one or both root compartments increased root growth (Table 2). This result is similar to previous studies (Li et al. 2016; Wu et al. 2016), and might be explained by the AM symbiosis enhancing carbon sink strength and carbon allocation by the host to cope with drought and nutrient uptake (Hodge 2004; Brunner et al. 2015). More than that, AM fungi also modifies root architecture to promote uptake of nutrient and water (Zhang et al. 2016, 2021; Zheng et al. 2020). However, there are also reports that the AM symbiosis did not improve root growth in split-root systems (Hao et al. 2012; Bárzana et al. 2015), and the different results might be due to the different experimental protocols.

Carbon assimilation of plants depends on photosynthesis of leaves (Lawlor and Cornic 2002). When *R. irregularis* was present in both root compartments, the photosynthetic parameters were improved (Table 1, Supplementary Table S3). This was in accordance with previous reports that AM fungi improve poplar photosynthesis when the poplar root was colonized (Liu et al. 2015; Li et al. 2015) and matched the biomass accumulation results. When plant photosynthesis is limited by drought stress, the electron leakage results in the reactive oxygen species (ROS) accumulation, and the scavenging of ROS depends on antioxidative enzymes and small molecular compounds (Ahmed et al. 2009; Gill and Tuteja 2010). The presence of *R. irregularis* in either one or two root compartments increased the activity of enzymes (except for SOD) and the concentrations of soluble protein, and decreased the concentrations of H_2O_2 and MDA (Table 1). This result is in accordance with the AM symbiosis improving photosynthetic parameters and increasing leaves RWC, and might be due to the AM symbiosis regulating plant genes involved in signal transduction and enzymes synthesis (He et al. 2017, 2020; Huang et al. 2020).

The absorption of phosphate from soils by roots requires participation of phosphate transporters from the PHT1 family (Nussaume et al. 2011). The presence of *R. irregularis* in root compartment upregulated the relative expressions of *PcPT3*, *PcPT4*, and *PcPT5* (Supplementary Table S5, Table 2). The expressions of *PcPT3*, *PcPT4*, and *PcPT5* orthologues in *Populus tremula* x *P. alba* were also upregulated by inoculation with *Glomus intraradices* or *G. mosseae* (Loth-Pereda et al. 2011). However, *PtPT3*, *PtPT4*, and *PtPT5* were not classified in the mycorrhizal inducible subfamilies, and their upregulation of expression might be involved in the transport of extra phosphate that uptake by the mycorrhizal pathway (Smith et al. 2011). Although the phosphorus concentration of poplar root was not affected by the presence of *R. irregularis* in neither one nor both root compartments, the phosphorus content of poplar root (data not shown) was increased due to the mycorrhizal effect on root DW accumulation. The improved phosphorus content also supported the transport of phosphate by the mycorrhizal pathway. Moreover, the expressions of *PcPT3*, *PcPT4*, and *PcPT5* were not affected by drought stress, and the concentration phosphorus was reduced by drought stress (Supplementary Table S5). This result resembles the expression of mycorrhizal-induced phosphate transporter genes and phosphorus uptake of *Lycium barbarum* under drought stress, and suggests the efficiency and independence of mycorrhizal Pi uptake regardless water status (Hu et al. 2016). The relative expression of *PcPT9* was downregulated by inoculation of *R. irregularis* and drought stress (Supplementary Table S5, Table 2). This might be due to the improved plant phosphate status of the AM symbiosis and the drought stress lowered plant phosphate demand by limiting growth, as its orthologues *PtPT9* in *Populus tremula* × *P. alba* and *OsPT9* in rice were induced by the low phosphate conditions (Loth-Pereda et al. 2011; Wang et al. 2014; Gan et al. 2015).

Aquaporins participate in water homeostasis in plants (Maurel et al. 2008). Drought stress downregulated the expressions of *PcPIP1-1*, *PcPIP2-1*, *PcPIP2-2*, and *PcPIP2-4* (Table 2), and this result indicated the responding of poplar to water deficiency by limiting water permeability in roots (Bárzana et al. 2014; Calvo-Polanco et al. 2016; Quiroga et al. 2017; Kilpeläinen et al. 2020). The presence of *R. irregularis* upregulated the relative expressions of *PcPIP2-3* and *PcPIP2-5*, and downregulated the relative expression of *PcPIP1-1* (Table 2). This result suggested an improved water permeability of poplar roots by *R. irregularis* as the PIP2s usually have higher water permeability than PIP1s (Chaumont et al. 2000; Bárzana et al. 2014; He et al. 2016), water transport by PIP2-5 has important contribution to poplar under water stress (Ranganathan et al. 2017) and the mycorrhizal-promoted transpiration. The relative expressions of TIPs were upregulated by the presence of *R. irregularis* in both root compartments (Table 2). The result suggests improved

cellular osmotic balance in AM fungal-colonized roots (Ruiz-Lozano 2003), and a possible involvement of TIPs in nutrient and H_2O_2 transport (Bienert et al. 2007; Bárzana et al. 2014; Quiroga et al. 2020).

In plant roots, ROS is generated through electron leakage and NADPH oxidization (Gill and Tuteja 2010; Marino et al. 2012). Drought stress increased the activity of antioxidative enzymes and concentrations of low molecular compounds independent of *R. irregularis* inoculation (Supplementary Table 7). This resembles previous studies, and fits the response of plants to balance ROS generation and scavenging (Wu et al. 2006; Bárzana et al. 2015; He et al. 2017). On the contrary, inoculation by *R. irregularis* decreased the activity of POD and CAT, but increased the activity of APX and concentration of soluble protein (Table 2). A possible explanation might be that the AM symbiosis improved plant water uptake as discussed above, and shifted plant roots from using enzymes to low molecular compounds that including soluble protein and ascorbates to scavenge ROS (Bárzana et al. 2015). Although the process of arbuscule development produce limited H_2O_2 (Belmondo et al. 2016), the presence of *R. irregularis* did not influence the concentration of H_2O_2 in roots (Table 2). This might be due to the limited colonization rate under drought stress (Fig. 2), the influence of drought stress on the antioxidative response of plant roots, and the influence of AM symbiosis on antioxidative responses in colonized root-system part (Bárzana et al. 2014; He et al. 2017).

In conclusion, our results indicated that inoculation by *R. irregularis* in either one or both compartments of the split-root system increased poplar biomass accumulation, photosynthesis, and ROS regulation under both well-watered and drought-stressed conditions. When inoculum was applied in both root compartments, the effect of *R. irregularis* was higher than that in treatment when only one compartment received inoculum. The effect of *R. irregularis* may be attribute to the improved phosphorus uptake via upregulation of relative expressions of *PcPT3*, *PcPT4*, *PcPT5*, and a possible improvement of water uptake via modulation of aquaporins in colonized root-system part. Further study directly compares the transcriptomic differences of AM fungal colonized and non-colonized root-system parts in the same poplar will find more AM fungi-regulated plant genes.

Author contribution statement HZ, LL, MT, and HC conceived and designed the study. HZ, LL, WREN, WZ conducted the experiments. HZ, LL, and WR analyzed the experimental data. HZ, LL, MT, and HC prepared and revised the manuscript.

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