Vertically transmitted Epichloë systemic endophyte enhances drought tolerance of Achnatherum inebrians host plants through promoting photosynthesis and biomass accumulation

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April 1, 2022

Abstract
Achnatherum inebrians (drunken horse grass, DHG) plants, a dominant grass species in the arid and semi-arid regions of northwest China, symbiotic with an Epichloë fungal endophyte, is well adapted to drought. However, little is known about how the presence of the foliar Epichloë endophyte enhances the tolerance of DHG to drought at the molecular level. This study explored the positive effects of the presence of the Epichloë endophyte on plant growth, biomass, and photosynthetic efficiency and processes of DHG under non-drought and two drought (moderate and severe) treatments, using RNA sequencing to compare transcriptomes. The transcriptome results showed that 32 selected unigenes involved in the photosynthesis processes within Epichloë symbiotic plants were differently expressed (DEGs) versus non-symbiotic plants. The majority of these selected DEGs were up-regulated in Epichloë symbiotic plants versus non-symbiotic plants, such as up-regulated unigenes (c51525.graph_c1, c47798.graph_c0 & c64087.graph_c0) under drought conditions. In line with the transcriptomes data, the presence of the Epichloë endophyte promoted the photosynthetic rate and biomass accumulation of DHG plants, and the relationship between photosynthetic rate and biomass is linear and significant. The presence of the endophyte only increased the biomass per tiller of DHG plants under drought. This study has provided further insights into the molecular mechanisms underlying enhanced plant growth and drought tolerance of DHG host plants of the Epichloë endophyte.

Introduction
Drought, a primary environmental factor, limits plant productivity in natural ecosystems (Ciais et al., 2005). In grasslands in the arid and semi-arid regions of northwest China, grasses including forage species in the family Poaceae are typically dominant species. Grassland species adapt and respond to drought through many strategies, such as changes of plant physical responses, biomass accumulation and/or allocation and accumulation of some protective metabolites (Cornelius et al., 2012; Hewitt et al., 2021). Natural selection and plant breeding can also enhance drought tolerance (Langridge & Reynolds, 2021). Some symbiotic beneficial microbes (e.g. root arbuscular mycorrhizal fungi and foliar Epichloë endophytes) enhanced plant drought tolerance through many strategies, such as absorbing water/nutrient through external hyphae of mycorrhizal and ectomycorrhizal fungi under drought conditions (de vries et al., 2020; Hewitt et al., 2021).

About 20-30% of grass species in the family Poaceae may be symbiotic with a foliar Epichloë fungal endophyte, promoting plant growth and enhancing persistence (Schardl et al., 2004; Gibert et al., 2019; Lee et al., 2021). Epichloë endophytes colonize the aboveground tissues of grasses but not the roots. Associations are generally symptomless and transmission in many associations is entirely vertical in nature, through the seed of host plants (Christensen et al., 2008). The presence of an Epichloë sp. can modify and enhance...
growth to avoid drought damage (Morse et al., 2002; Schardl et al., 2004; Decunta et al., 2021; Xu et al., 2021). Further, the presence of the Epichloë endophyte produces or induces the production of some bioactive metabolites (fungal alkaloids and phytohormone) to help the host plant to adapt to drought (Schardl et al., 2004; Nagabhryut et al., 2013; Decunta et al., 2021; Zhao et al., 2021). The presence of an Epichloë sp. also regulates the plant physical responses to reduce/eliminate plant damage from water deficient, such as through stomata regulation, osmotic adjustment and enhanced water use efficiency (WUE) (Elmi & West, 1995; Xia et al., 2018a; Decunta et al., 2021). Models predict that crop yield can be improved through enhancing efficiency of the photosynthetic process, and photosynthetic efficiency can be used to predict plant drought tolerance (Ort et al., 2015). Epichloë symbiotic plants have been reported to have higher net photosynthetic efficiency and biomass accumulation than non-symbiotic plants (Spiering et al., 2003; Rozpaδek et al., 2015; Zhang et al., 2018), and this knowledge led us to assess if this enhancing effect was linked to drought tolerance. We accessed effects of the presence of an Epichloë endophyte in host grasses on net photosynthetic rate through a standard meta-analysis based on published procedures.

Achnatherum inebrians (drunken horse grass, DHG) plants symbiotic with either Epichloë gansuensis or E. inebrians, are widely distributed in the arid and semi-arid regions of China, and the Epichloë infection rate of wild DHG plant populations could reach 100% (Nan & Li, 2000; Li et al., 2004; Chen et al., 2015). DHG plants have the potential to be utilized as an animal feed because of high levels of crude proteins (close to that of Medicago sativa), in addition to the fact that DHG plants without the Epichloë endophyte do not cause disorders to grazing livestock (Liang et al., 2017). E. gansuensis symbiotic DHG plants (EI) exposed to drought had greater plant biomass/nutrient accumulation and faster photosynthetic efficiency than those DHG plants without the endophyte (EF) in controlled pot experiments and in field trials in semi-arid regions (Zhang et al., 2015; Xia et al., 2016; 2018). Additionally, Epichloë symbiotic DHG plants had important ecological roles in plant competition and pathogen control in natural grassland ecosystem (Yao et al., 2016; 2020). Here we studied the tolerance of Epichloë associated DHG plants against drought stress. We subjected Epichloë sp. symbiotic and non-symbiotic DHG plants to water restriction treatments (i.e., severe, moderate, no drought). We hypothesized that plants associated with the Epichloë sp. may exhibit high tolerance to the drought due to the Epichloë-mediated enhancement of the host plant photosynthesis. For this undertaking, we measured plant growth variables (as proxies of tolerance to the stress), and the photosynthetic capacity in EI and EF plants. In addition, we identified the specific photosynthesis-related genes linked to the variations in photosynthesis rates.

**Material and methods**

**Seeds origin, Plant materials & Experimental design**

Seeds of E. gansuensis symbiotic DHG plants were generated from one grass population collected from the grassland in Sunan County, Gansu, China (101°01’ E, 38°35’ N, attitude 3297 m). Epichloë non-symbiotic DHG plants were generated by treating symbiotic seeds from F0 generation with a systemic fungicide (Thiophanate-methyl, 70% effective component) with 100 times dilution and 2 h treatment (Li et al., 2016). In order to multiply seeds, fungicide-treated and untreated seeds were planted in contiguous plots in an experimental field of Yuzhong campus of Lanzhou University. All DHG plants were checked by microscopic examination for the presence of seldom-branched hyphae characteristic of Epichloë spp., in leaf sheath pieces stained with aniline blue and also in seeds of individual plants, and plants were individually labeled as EI or EF plants respectively. Additionally, we did not observe effects of fungicide treatment on the morphology, phenology, and growth of our experimental plants through pot (Li et al., 2016) and field (Zhang et al., 2015; Zhong et al., 2021) experiments. Seeds were collected from EI and EF DHG plants grown in the experimental field, and stored at -4°C before planting.

Three EI or EF seeds were planted into one plastic pot (diameter: 24 cm, height: 15 cm) filled with 200 ± 2 g sterilized vermiculite (120°C for 5 h), and later thinned to one seedling per pot. Hoagland’s solution was
used to quantitatively water these experimental pots every other day after the appearance of the second fully expanded leaf of individual plants (Xia et al., 2016) [26]. Pots were maintained in a constant-temperature (26 ± 2°C) greenhouse. After one month, pots containing similar sized EI (n = 27) and EF (n = 27) seedlings were cut 15 cm above the vermiculite surface, and the water-holding capacity of each pot was reduced to 15% relative saturation moisture content (RSMC). Subsequently, severe drought (SD, 15% RSMC), moderate drought (MD, 30% RSMC) and no drought (CK, 60% RSMC) treatments were established and sustained for 50 days.

Differentially expressed genes

At the end of the soil moisture treatments, three fresh leaves of three EF or EF DHG plants for each soil moisture treatment were collected and immediately frozen in liquid nitrogen, and then stored at -80°C for the subsequent RNA extraction and transcriptome sequencing (supplementary files). Transcriptome analysis in this present study was performed by Biomarker Technologies (Beijing, China). A total amount of 3 μg RNA per sample was used as input material for the preparations of RNA samples. Sequencing libraries were generated using the NEBNext® Ultra RNA Library Prep Kit for Illumina(r) (NEB, USA). Afterwards, the libraries were sequenced by the Illumina HiSeq 2000 platform. The function of these unigenes was annotated based on these following databases:

NCBI Non-redundant protein sequences (NR,ftp://ftp.ncbi.nih.gov/blast/db/);
Protein family (Pfam,http://pfam.xfam.org/);
Clusters of Protein Homology (KOG,http://www.ncbi.nlm.nih.gov/KOG/);
Clusters of Orthologous Groups of proteins (COG,http://www.ncbi.nlm.nih.gov/COG/);
Orthologous groups of genes (eggNOG,http://eggno gdb.embl.de/);
A manually annotated and reviewed protein sequence (Swiss-Prot,http://www.uniprot.org/);
Kyoto Encyclopedia of Genes and Genomes (KEGG,http://www.genome.jp/kegg/);
Gene Ontology (GO,http://www.geneontology.org/).

Gene expression levels were estimated on the basis of fragments per kilobase of transcript per million mapped fragments (FPKM) by RSEM for each sample (Li and Colin, 2011). Clean data were mapped back onto the assembled transcriptome; read count for each gene was obtained from the mapping results. For each treatment, with three biological replicates, differential expression analysis of the two groups (the EF plants were as the control group) was performed using the DESeq2 package in R software (version 1.10.1) which provides statistical routines for determining differential expression of genes using a model based on the negative binomial distribution. The resulting P values were adjusted using the Benjamini and Hochberg’s approach for controlling the false discovery rate (FDR) at 0.05. These log2 [fold changes (FC)] of unigene FPKM were used to identify whether these unigene were differentially expressed genes (DEGs) (Storey, 2003). We used KOBAS2.0 software to test the statistical enrichment of DEGs in KEGG pathways (Mao et al ., 2005; Kanehisa et al ., 2008). DEGs involved in photosynthesis (ko00195) and photosynthesis-antenna proteins (ko00196) from the present transcriptomic data were selected for the further analysis. Here, a total of 32 unigenes were selected for the subsequent analysis.

Amino acid sequences of these selected unigenes were blasted (blastx) against the genome of a related species to get the referenced sequences, and the resulting and reference sequences were used to construct Neighbor-Joining (NJ) phylogenetic trees using Molecular Evolutionary Genetics Analysis (MEGA, version 10.0.5) software (Kumar et al ., 2018). Total leaf RNA of each sample used for RNA-Seq analysis was also used for quantitative real-time PCR (qRT-PCR) analysis. Single-stranded cDNAs were synthesized from 2.5 μg of total RNA with MMLV reverse transcriptase (TaKaRa, Dalian, China). The qRT-PCR was performed using SYBR Premix Ex Taq II Kit (TaKaRa, Dalian, China) on a 7500 Fast Real-time PCR
system (Applied Biosystems, USA). The specific primers sequences of these selected unigenes used in the present study are listed in the Table S1. Three technical replicates were carried out for each reaction, and the relative expression levels were normalized to the expression of the unigene (ID: c56016.graph_c0 detected in the present study) and calculated using the $2^{-\Delta\Delta T}$ method.

Indexes of plant growth and photosynthesis

In order to test the tolerance of *Epichloë* symbiotic DHG plants to severe and moderate drought, we assessed the indices of plant growth (plant height, tiller number and biomass), chlorophyll content and photosynthesis (photosynthetic rate, intercellular carbon dioxide (CO$_2$) concentration, stomatal conductance and transpiration rate). The heights and tiller numbers of EI and EF DHG plants were measured at the completion of the soil moisture treatments. The effects of the presence of the *Epichloë* endophyte on the photosynthetic rate of host plants were obtained through a standard meta-analysis (see the detail information in the supplementary).

The chlorophyll content of three leaves of one individual EI or EF DHG plant was measured using a chlorophyll meter (SPAD-502Plus, Konica Minolta Sensing, Inc., Japan), then the mean of three measurements represented the actual value of this individual plant. The photosynthetic indexes were performed using a portable photosynthesis system (LI-6400, LI-COR Nebraska, USA) between 9:00 and 11:00 at the morning of the final day of soil water treatments. The concentration of air CO$_2$ was 410 ± 10 μmol CO$_2$ mol$^{-1}$, the chamber was equipped with a red/blue LED light source (LI6400-02B), with the photo flux density set at 1200 μmol m$^{-2}$ s$^{-1}$, and the detesting conditions were at 28 ± 1°C. Finally, the shoots and roots of sampled plants were collected from the nine pots to measure the fresh weight of shoots and roots, and then the dry weight of shoots and roots were recorded when a constant weight had been reached in an 80°C oven for 48 h.

Data analysis of plant parameters

Differences in plant growth performance, biomass and photosynthetic index under the *Epichloë* endophytic status and different soil moisture levels were tested using a two-way analysis of variance (ANOVA) using the datarium package of R software. A statistically significant two-way interaction was followed up by simple main effect analyses; that is, evaluating the effect of endophytic status at each soil moisture treatment. All values are means ± SE of the mean.

Results

Differentially expressed genes in photosynthesis

A total of 462911295 clean reads were obtained from all samples, and 64.88%-70.95% reads of each sample were mapped and used for further analysis (Table S2). A total of 92964 unigenes were detected from 18 samples with the mean length for all unigenes being 865.18 bp, with an N50 length of 1677 bp (Table S3). A total of 42618 (45.85%) unigenes were annotated in eight different public databases including COG (13.27%), GO (27.22%), KEGG (13.40%), KOG (24.20%), Pfam (29.05%), Swissprot (22.20%), eggnog (41.95%) and Nr (39.62%) databases (Table S4). The results indicated that expression of all unigenes differed between EI and EF plants regardless of non-drought and drought treatments (Figure 1A & S1). Few DEGs in DHG plants in the drought (SD: 116 DEGs and MD: 11 DEGs) treatments versus CK treatment were detected (Figure S2). There were 1349 (680 up and 669 down), 1119 (382 up and 737 down) and 591 (297 up and 294 down) DEGs in EI DHG plants versus EF plants under CK, MD and SD treatments, respectively (Figure 1B).

KEGG pathways (top 20) results indicated that these DEGs involved in the photosynthesis processes responded to the presence of the *Epichloë* endophyte in the CK treatment, such as for photosynthesis (ko00195),
antenna proteins (ko00196), chlorophyll metabolism, carbon fixation in photosynthetic organisms and other metabolites processes (Figure 1C). Similar unigenes of these DEGs are also reported in some crop and model plants in the sub-family Pooidae. The identity of these DEGs with reference genes was over 80%, from 80.18% to 100% (Table 1). There were 16 DEGs associated with the process of photosynthesis, such as photosystem II (8 DEGs: \( \text{psbB, psbC, psbE} \), two psbS, \( \text{psbQ and psbB27} \) ), photosystem I (3 DEGs: \( \text{psaO, psaE and psaG} \) ), photosynthetic electron transport (5 DEGs: \( \text{petE, petF, two petH and petJ} \) ) and F-type ATPase (1 DEG: \( \text{atpH} \) ) (Figure 1D). There were 16 DEGs identified that were associated with the process of photosynthesis-antenna proteins, including \( \text{lhcB1 (10), lhcB2 (2), lhcB3 (1) and lhcB5 (2) and lhcB6 (1)} \) unigenes (Table 1 & Figure S3).

Significant positive linear relationships between transcriptome data and qRT-PCR were observed in CK (\( R = 0.648, P < 0.001 \) ), MD (\( R = 0.588, P < 0.01 \) ) and SD (\( R = 0.599, P < 0.01 \) ) treatments, respectively (Figure 2ABC). The majority of selected DEGs in photosynthesis and antenna proteins were up-regulated in EI DHG plants versus EF DHG plants under CK, MD & SD treatments, respectively (Figure 2D). DEGs (e.g. \( c51525, c47798, c64087 \) ) were only up-regulated in the EI versus EF DHG plants under drought conditions (Figure 2BCD). \( \text{Epichloé} \) presence up-regulated three DEGs (\( c47622, c56765, c54664 \) ) expression regardless of non-drought and drought treatments (Figure 2). Meanwhile, the majority of DEGs were up-regulated in CK and SD treatments (Figure 2ACD).

**Photosynthesis**

As the consequence of the upregulation of the majority of photosynthesis DEGs, we accessed whether photosynthetic rates were higher in EI versus EF DHG plants. Two-way ANOVA results indicated that plant chlorophyll content and photosynthetic indices differently responded to the drought treatments and the \( \text{Epichloé} \) presence (Table 2 & Table S5). The effects of the presence of the \( \text{Epichloé} \) endophyte on leaf chlorophyll content depended on the soil moisture, and symbiosis \( x \) soil moisture treatments: \( F_{(2,48)} = 15.73, P = 0.000 \), Table 1. The \( \text{Epichloé} \) presence significantly increased the leaf chlorophyll content only in the MD and SD treatments in 19.7% (\( P = 0.000 \) ) and 7.1% (\( P = 0.040 \) ), respectively (Figure 3A). The chlorophyll content in EI DHG plants was only significantly higher than in EF DHG plants under MD & SD treatments (Figure 3A). The MD & SD reduced the photosynthetic efficiency of DHG plants compared to the CK level of soil moisture: \( F_{(2,48)} = 47.80, P < 0.001 \), Figure 3B. \( \text{Epichloé} \) presence increased the photosynthetic efficiency of DHG plants regardless of soil moisture treatments, symbiosis status: \( F_{(1,48)} = 14.08, P < 0.001 \), with increases of 17.2% (\( P = 0.000 \) ), 10.7% (\( P = 0.022 \) ) and 10.9% (\( P = 0.030 \) ) under CK, MD and SD treatments, respectively (Figure 3B). The intercellular carbon dioxide concentration was significantly higher in EI versus EF DHG plants under two drought conditions, with an increase of 19% and 22% under MD & SD, respectively (Figure S4A). The transpiration rate was only significantly lower in EI versus EF plants under non-drought conditions (Figure S4C).

**Plant growth and Biomass**

The plant performance and shoot/root/total biomass significantly responded to the presence of the \( \text{Epichloé} \) endophyte and drought treatments (Table 1, Table S5 & Figure S5). The effect of \( \text{Epichloé} \) on plant height depended on the soil moisture, symbiosis \( x \) soil moisture treatments: \( F_{(2,48)} = 17.27, P = 0.000 \), Table S5. The \( \text{Epichloé} \) endophyte significantly increased the plant height only in the MD and SD treatments by 13.1% (\( P = 0.000 \) ) and 9.6% (\( P = 0.000 \) ), respectively (Figure S1A). The effects of the \( \text{Epichloé} \) endophyte on total biomass depended on the soil moisture, symbiosis \( x \) soil moisture treatments: \( F_{(2,48)} = 7.67, P = 0.001 \), Table S5. In line with the results of shoot/root fresh/dry weight (Figure S5ABCD), the \( \text{Epichloé} \) endophyte significantly increased the total biomass in the CK, MD and SD treatments in 12.7% (\( F_{(1,16)} = 288.0, P = 0.000 \) ), 11.3% (\( F_{(1,16)} = 58.9, P = 0.000 \ ) ) & 21.4% (\( F_{(1,16)} = 88.1, P = 0.000 \ ) ) respectively (Figure 3C). The drought treatments significantly decreased tiller number of DHG plants compared with CK treatments regardless of symbiosis status, \( F_{(2,48)} = 17.27, P = 0.000 \), Figure 3D. Here, the present results indicated that the \( \text{Epichloé} \) endophyte only had significant positive effects on the average per-tiller biomass under the MD
and SD treatments (Figure 3E). Additionally, the total biomass of EI and EF DHG plants was significantly (P < 0.001) and positively associated with the photosynthetic efficiency regardless of the presence or absence of the *Epichloe* endophyte (Figure 3F).

**Meta-analysis**

There were positive effects of the *Epichloe* endophyte on net photosynthetic efficiency (NPE) (effect size = 0.102, 95% CI = 0.057 to 0.148), water use efficiency (effect size = 0.128, 95% CI = -0.027 to 0.283) and photochemical efficiency (effect size = 0.011, 95% CI = -0.060 to 0.082) of EI plants than EF plants through this present meta-analysis (Figure 4). There was an overall positive effect (main effect size = 0.101, 95% CI = 0.077 to 0.125) of the *Epichloe*endophyte on NPE of EI plants compared to EF plants (Qb = 98.2, P = 0.000, df = 10) (Figure 4). The higher NPE were found in DHG (effect size = 0.170, 95% CI = 0.115 to 0.226), *A. sibiricum* (effect size = 0.034, 95% CI = 0.032 to 0.099), *Calamagrostis epigeios* (effect size = 0.122, 95% CI = -0.207 to 0.451), *Festuca sinensis* (effect size = 0.249, 95% CI = 0.132 to 0.367), *Hordeum brevisubulatum* (effect size = 0.171, 95% CI = 0.095 to 0.246), *Lolium perenne* (effect size = 0.147, 95% CI = 0.094 to 0.200), *Stipa purpurea* (effect size = 0.165 95% CI = 0.039 to 0.291) and *F. arundinacea* (effect size = 0.102, 95% CI = 0.015 to 0.189) plants infected by *Epichloe* endophyte (Figure 4). While, the lower NPE were only observed in the *F. arizonica* (effect size = 0.214, 95% CI = -0.297 to -0.131) and *Elymus tangutorum* (effect size = 0.040, 95% CI = -0.169 to 0.088) plants infected by *Epichloe* endophyte (Figure 4).

**Discussion**

With plants exposed to water deficit, production was linked to the net photosynthetic efficiency. Adaption mechanisms of plants to abiotic and biotic stresses include the forming of symbiotic associations with beneficial microbes. In this study that we conducted into the effects of different levels of drought stress on *A. inebrians* plants with and without an *Epichloe* systemic endophyte our results provide a comprehensive overview of unigenes changes associated with photosynthesis processes (Figure 5). We found that the majority of DEGs in photosynthesis were up-regulated in *Epichloe* symbiotic plants and thus had a higher NPE than non-symbiotic plants (Figure 5).

Many studies have confirmed that the presence of an *Epichloe* endophyte promoted plant growth, biotic resistance and abiotic tolerance of their host grasses (Bastias et al., 2017; Xia et al., 2018b; Hewitt et al., 2021; Lee et al., 2021). Studies have provided an understanding of how symbiotic microbes improved drought tolerance through different strategies (Swarthout et al., 2009; de vries et al., 2020; Decunta et al., 2021). The secondary metabolites produced or induced by the presence of an *Epichloe* endophyte, contribute to the plant abiotic/biotic tolerance (Bastias et al., 2017; Xia et al., 2018b). Plant metabolites processes begin with the products of the photosynthesis process. The presence of an *Epichloe* endophyte in grasses stimulates the accumulation of plant metabolites in the above ground tissues and in roots, such as SA, flavonoids and total phenolic compounds (Ponce et al., 2009; Bastias et al., 2017; Hou et al., 2021). As we expected, the unigenes in flavonoids and fatty acids biosynthesis were differently expressed in response to the *Epichloe* endophyte.

The presence of *Epichloe* spp. in aboveground tissues altered the transcription levels of their host cool-season grasses (Khan et al., 2010; Chen et al., 2016; Dinkins et al., 2017; 2019). The expression of dehydrin and heat shock protein genes in *F. arundinacea* was enhanced by the symbiotic *Epichloe* endophyte in water-unstressed conditions (Dinkins et al., 2019). Similarly, the majority of unigenes that differently responded to the *Epichloe* presence were found in the control, abiotic and biotic conditions, such as those involved with SA biosynthesis (Kou et al., 2021; Zhao et al., 2021). As expected, this present study also detected a large number of DEGs in EI plants versus EF DHG plants under the three soil moisture treatments, and these DEGs detected in the present transcriptome data included some involved in photosynthesis (PSI, PSII and
PET), in line with our hypothesis (Figure 5). Photosynthesis begins with harvesting light within leaves, and the present transcriptome data indicated that genes in antenna proteins and the chlorophyll metabolism process were up-regulated in response to the *Epichloë* endophyte, and a higher chlorophyll content was found in EI versus EF DHG plants. These present results are also supported by two studies (Ambrose and Belanger, 2012; Rozpadek *et al*., 2015). The abundance of lhcI and lhcII proteins in EI *D. glomerata* plants was higher that of EF plants (Rozpadek *et al*., 2015). Meanwhile, the genes of lhcI type II were upregulated in the *E. festucae*-infected *F. rubra* compared with EF plants (Ambrose and Belanger, 2012).

Ambrose and Belanger (2012) also noted that some genes involved in the photosynthesis process of red fescue (*F. rubra*) are upregulated and downregulated to respond to the presence of an *Epichloë*endophyte. The *Epichloë* endophyte increased the rate of carbon assimilation, PSII photochemistry and grass biomass associated with *D. glomerata* plants (Rozpadek *et al*., 2015). A study showed that the 1000 D7 gene (CP47) was down-regulated in perennial ryegrass symbiotic with *E. festucae* var. *loli* (Khanet *et al*., 2010). Similar to that study of *Epichloë* symbiotic perennial ryegrass, our results indicated that a unigene (c61885.graph_c1, encored PSII CP47 reaction center protein) was down-regulated in the EI DHG plants regardless of non-drought and drought treatments. Another study found that the electron transport rate was enhanced 31% in EI plants and reduced 13% in EF plants under water stressed versus CK treatments (Amalric *et al*., 1999). As we expected, one (c51525.graph_c1, petH) and two (c47702.graph_c0,petF; c46095.graph_c0,petJ) up-regulated unigenes were only in EI DHG plants, under drought and non-drought treatments, respectively. This is in line with a study that indicated that the NADPH activity in EI *D. glomerata* plants was significantly greater (c. 4.28) than EF plants (Rozpadek *et al*., 2015). Our results and present meta-analysis indicated that the *Epichloë* endophyte promoted the photosynthetic rate of host plants (Figure 5). Meanwhile, the presence of the *Epichloë* endophyte on net photosynthesis rate of *L. perenne* is independent of endophyte concentration *in planta* (Spiering *et al*., 2006).

Greater photosynthetic rates are commonly associated with higher production, and our results showed the liner relationship between photosynthetic rates and total biomass of EI and/or EF DHG plants. In line with our hypothesis and some studies, the presence of an *Epichloë* endophyte increased the biomass and photosynthetic rate of host plants under drought (Zhang *et al*., 2015; Xia *et al*., 2016; 2018b; Xu *et al*., 2021). Another study found that EIF. arizonica plants produced more shoot biomass and had greater plant growth rate versus EF plants under low water availability (Morset *et al*., 2002). Our results showed that the presence of the *Epichloë* endophyte had no effects on the per-tiller biomass under non-drought treatment, while promoted the per-tiller biomass accumulation under (MD & SD) drought conditions. This study provides us with an enhanced understanding of the enhancement of drought tolerance in *Epichloë* symbiotic plants (Figure 5).

**Acknowledgements**

Thanks for the Daniel Bastias, Michael Christensen and Richard Johnson from AgResearch Ltd, Grasslands Research Centre, New Zealand for their valuable suggestions. This work was financially supported by the National Nature Science Foundation of China (31772665).

**Declaration of Competing Interest**

The authors declare no conflict of interest.
Author contributions

R.Z. and X.X.Z. designed the experiment, R.Z. conducted the experiments and analysed the data, R.Z. and X.X.Z. wrote this manuscript; and all authors revised this manuscript.

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**Figure**

**Figure 1.** The unigenes and differently expressed genes (DEGs) based on the leaf transcriptome data of Achnatherum inebrians plants in response to the presence of the Epichloë fungal endophyte under non-drought (CK), moderate (MD) and severe (SD) drought treatments. **Note:** A: The sample similarity analysis of the all unigenes detected in the leaves of Epichloë symbiotic (EI) and non-symbiotic (EF) plants under CK, MD and SD moisture treatments. B: The number of all and up/down-regulated DEGs in EI plants versus EF plants under CK, MD and SD moisture treatments. C: Kyoto Encyclopedia of genes and genomes (KEGG) pathway enrichment of DEGs in response to the Epichloë endophyte under CK treatment.

**Figure 2.** Validation of the expression changes (log$_2$(fold change, FC)) and heatmap (FC>1.5, plus) of selected genes involved in the processes of photosynthesis and photosynthesis-antenna proteins from RNA-Seq using qRT-PCR under normal (CK, A), moderate drought (MD, B) and severe drought (SD, C) moisture treatments. **Note:** The results are plotted for genes that show significant upregulation and down regulation of leaves in response to the fungal endophyte under different soil moisture levels (D). The linear trend line, the R-value and 95% confidence interval are shown.

**Figure 3.** The chlorophyll content (A), photosynthetic rate (B), total biomass (C) and The tiller number (D) of Epichloë symbiotic (EI) and non-symbiotic (EF) plants under normal (CK), moderate drought (MD) and severe drought (SD) treatments, and liner linear regression (F) between photosynthetic rate and total biomass. **Note:** Different lowercase letters mean significant difference at P<0.05 among three soil moisture treatments at 0.05 level. The * means significant difference at 0.05 between and EI and EF plants at corresponding water content at 0.05 level.

**Figure 4.** Effects of symbiotic Epichloë fungal endophyte on the net photosynthetic efficiency (NPE), water use efficiency (WUE) and photochemical efficiency (Fv/Fm) of grasses (Relative effects of Epichloë symbiotic versus non-symbiotic plants).

**Figure 5.** The overview diagram for understanding the advantages of Epichloë symbiotic Achnatherum inebrians plants versus non-symbiotic plants combining the transcriptomes, photosynthesis and growth.

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