Microbial Ecology (2024) 87:73
https://doi.org/10.1007/s00248-024-02391-2

RESEARCH

Endophytes Alleviate Drought-Derived Oxidative Damage in *Achnatherum inebrians* Plants Through Increasing Antioxidants and Regulating Host Stress Responses

Xiumei Nie[1] · Zhenrui Zhao[1] · Xingxu Zhang[1] · Daniel A. Bastías[2] · Zhibiao Nan[1] · Chunjie Li[1]

Received: 24 January 2024 / Accepted: 9 May 2024
© The Author(s) 2024

Abstract
Endophytes generally increase antioxidant contents of plants subjected to environmental stresses. However, the mechanisms by which endophytes alter the accumulation of antioxidants in plant tissues are not entirely clear. We hypothesized that, in stress situations, endophytes would simultaneously reduce oxidative damage and increase antioxidant contents of plants and that the accumulation of antioxidants would be a consequence of the endophyte ability to regulate the expression of plant antioxidant genes. We investigated the effects of the fungal endophyte *Epichloë gansuensis* (C.J. Li & Nan) on oxidative damage, antioxidant contents, and expression of representative genes associated with antioxidant pathways in *Achnatherum inebrians* (Hance) Keng plants subjected to low (15%) and high (60%) soil moisture conditions. Gene expression levels were measured using RNA-seq. As expected, the endophyte reduced the oxidative damage by 17.55% and increased the antioxidant contents by 53.14% (on average) in plants subjected to low soil moisture. In line with the accumulation of antioxidants in plant tissues, the endophyte increased the expression of most plant genes associated with the biosynthesis of antioxidants (e.g., *MIOX*, *crtB*, *gpx*) while it reduced the expression of plant genes related to the metabolization of antioxidants (e.g., *GST*, *PRODH*, *ALDH*). Our findings suggest that endophyte ability of increasing antioxidant contents in plants may reduce the oxidative damage caused by stresses and that the fungal regulation of plant antioxidants would partly explain the accumulation of these compounds in plant tissues.

Keywords *Epichloë* · Plant stress tolerance · Soil moisture · Oxidative stress · Reactive oxygen species · Plant transcriptomes

Introduction

Plants inevitably withstand various environmental stresses during their lifetime. Drought has become one of the most important limiting factors in global agricultural production, with losses estimated in around $30 billion in the last decade [1]. Under the current global warming, drought events are predicted to become more frequent and the aridity is more severe in most semi-arid and arid areas [2]. The association of plants with beneficial endophytic fungi generally enhances the plant resilience to harsh environments [3–5]. Poöideae (family Poaceae) grasses that form associations with beneficial and asexual fungal endophytes of the genus *Epichloë* are examples of this [6, 7]. These endophytes promote changes in plant morphology, physiology, and biochemistry that increase the host resistance/tolerance to stresses including drought (e.g., root architecture, stomata regulation, and antioxidant contents) [6, 8, 9].

The perennial bunchgrass *Achnatherum inebrians* (Hance) Keng that is widely distributed in arid and semi-arid regions of northwestern China forms symbiotic relationships with either *Epichloë gansuensis* (C.J. Li & Nan) or *Epichloë inebrians* (C.D. Moon & Schardl) [7, 10, 11]. Compared to sexual endophytes, asexual *Epichloë* endophytes generally form mutualistic relationships with their

✉ Xingxu Zhang
xxzhang@lzu.edu.cn

✉ Daniel A. Bastías
daniel.bastias@agresearch.co.nz

[1] State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China

[2] Grasslands Research Centre, AgResearch Limited, Palmerston North 4442, New Zealand

Published online: 17 May 2024

hosts, asymptomatically colonize plant foliar tissues, and are maternally inherited through mycelia established in mature seeds [12, 13]. *Epichloë* spp. associated with *A. inebrians* plants usually enhance the host tolerance and resistance to stresses [14–16]. In drought situations, these endophytes increase the plant tolerance to the stress through promoting the host seed germination, biomass, nutrient acquisition, and photosynthetic rate [17–19]. Additionally, the *E. gansuensis*–mediated drought tolerance in *A. inebrians* plants is partially attributed to the endophyte's ability to regulate the expression of plant genes involved in the biosynthesis hormones and cuticular wax [20, 21].

Reactive oxygen species (ROS) are byproducts of aerobic metabolic processes in living organisms and include superoxide anions (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) [22]. Excessive accumulation of ROS in cells can cause oxidative stress, which damages DNA, lipids, and proteins, disrupting normal metabolism and leading to cell death [23]. Oxidative damage in cells can be determined by quantifying the concentration of malondialdehyde (MDA) compounds that are generated in the ROS reactions with lipids [24]. The accumulation of ROS in cells can be prevented by the presence of enzymatic and non-enzymatic antioxidants. These compounds efficiently scavenge ROS and in stressful conditions substantially minimize the potential damage caused by ROS to plant cells [25]. Enzymatic antioxidants include catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), while non-enzymatic antioxidants include ascorbic acid (AsA), carotenoid (Car), glutathione (GSH), and proline (Pro) [22, 26].

Epichloë endophytes usually increase the contents of antioxidants in plants experiencing environmental stresses [27, 28]. In drought stress, *Epichloë* spp. have been shown to enhance the plant contents of distinct enzymatic and non-enzymatic antioxidants including ascorbate peroxidase (APX), CAT, glutathione reductase (GR), and Pro [29, 30]. Similar endophyte-based accumulation of antioxidants has been documented in *A. inebrians* plants exposed to distinct environmental stresses [31, 32]. The accumulation of antioxidants in endophyte-infected plants under stressful conditions may counteract the oxidative damage caused by the stresses and ultimately increase the plant fitness [9, 27]. In fact, the increased antioxidant contents documented in *Epichloë*-infected *Elymus dahuricus* plants under drought stress were correlated with reduced oxidative damage and increased plant biomass [33]. Although not entirely understood, the ability of *Epichloë* to stimulate the accumulation of antioxidants in plant tissues would be associated with the endophyte regulation of the plant production of antioxidants and/or the fungal production of these compounds [34, 35]. For instance, the expression of the plant genes *CsFe-SOD*, *CsMn-SOD*, *CsCAT1*, and *CsPRR7* associated with SOD and CAT pathways in *Citrus sinensis* was increased by the association with distinct beneficial fungi (e.g., *Acaulospora scrobiculata, Piriformospora indica*) [36]. Similarly, the inoculation of *Funneliformis mosseae* mycorrhizal fungi in *Poncirus trifoliata* increased the expression of the plant genes *PtMn-SOD*, *PtCAT1*, and *PtPOD* also associated with SOD and CAT pathways in response to drought stress [37].

Here, we evaluated the oxidative damage and antioxidant responses of plants associated with *Epichloë* endophytes subjected to contrasting soil moisture conditions. Plants of *A. inebrians* infected with (EI) and free (EF) of the endophyte *E. gansuensis* were grown in low and high soil moisture conditions. The levels of oxidative damage, contents of antioxidants, and the expression of plant genes coding for enzymes associated with antioxidant pathways were assessed. We hypothesized that the presence of endophytes in plants would simultaneously reduce the oxidative damage and increase the contents of antioxidants, especially at low soil moisture. We also hypothesized that the endophyte effects on the expression of plant genes associated with antioxidant pathways would be aligned with changes in the patterns of accumulation/reduction of antioxidants across soil moisture conditions.

Materials and Methods

Plant Material and Experimental Treatments

A few leaf sheaths of around 100 individual *A. inebrians* plants (with 15–30 tillers) were harvested, stained with aniline blue, and examined by microscope (× 40, Leica DMRB, Germany) in 2011. Almost all these plants were infected with the endophyte. Seeds were collected from the EI plants, and a subset of 50 seeds per plant were randomly selected to determine the endophyte infection through microscopy using a similar protocol to that used for leaf sheaths [10]. The endophyte was present in all seeds (50 out of 50 per EI plant). Seeds from EI plants were pooled together and labeled as the F0 seed lot. To obtain seeds free of *E. gansuensis*, half of the seeds from F0 seed lot were treated for 2 h with the fungicide thiophanate-methyl (70%, diluted 100 times) and the other half were not treated with any fungicide [38]. To obtain stocks of EI and EF seeds for experimentation, 200 seedlings from fungicide-treated seeds and 200 seedlings from untreated seeds were sown separately in the experimental field at the College of Pastoral Agricultural Science and Technology located in the Yuzhong Campus of Lanzhou University (Lanzhou, China; 104° 39′ E, 35° 89′ N, altitude 1653 m) in 2012. The seedling endophyte status was checked by microscopy on leaf sheaths following the same protocol above mentioned, and plants from fungicide-treated and non-treated seeds were 0% and 100% infected

with the endophyte respectively. Seeds produced from these two plant groups were labelled as EF and EI, respectively. Seed increases from this original source of EF and EI seeds are produced every year in our facilities, and the contrasting endophyte infection rate has been maintained across generations (0% and 100%, respectively).

A pot experiment was conducted in a greenhouse with constant temperature (temperature 26 ± 2 °C, humidity 42 ± 2%) for 3 months at the College of Pastoral Agricultural Science and Technology in 2020. Fresh seeds were obtained from the 2019 seed increase (recall that seed increases have been produced every year since the generation of EF and EI seeds in 2012). The soil substrate used in the experiment was a combination of peat soil and vermiculite (2:1 volume), sterilized at 180 °C for 2 h in an oven. A total of 200 plastic pots (upper diameter 12 cm, lower diameter 10 cm, height 13.5 cm, weight 30 g) lined with filter papers of 90 mm of diameter at the bottom were filled with 250 g of the sterilized soil substrate. Seeds of *A. inebrians* were sown in the 200 pots (100 pots for EI plants and 100 pots for EF plants), with three seeds per pot. The endophyte infection status was checked by microscopy in a single tiller per plant and confirmed that all EI plants were infected with the endophyte, while all EF were free of the endophyte. The pots were then watered daily according to the water-holding capacity (WHC) of each pot, maintaining 30% of soil moisture content as determined from the relative saturated moisture content (RSMC) at the start of the experiment. The RSMC represents the ratio of the actual soil moisture content to the potential soil moisture saturation, both determined by measuring the moisture contents in 10 pots [17]. In this experiment, 30% soil moisture was chosen as the control condition, since this moisture was the threshold that prevented plants entering in wilting point when watered at intervals of 24 h [19]. The positions of pots were changed every 3 days in the greenhouse. A month post sowing, most seedlings showed second true leaves, and a single plant per pot was retained based on similar size. The retained plants were watered with half-strength Hoagland nutrient solution once a week for 4 weeks.

After 2 months of growth, the watering of plants was ceased, and pots were weighed until the WHC of each pot reached a soil moisture content of 15% according to the RSMC. At this point, a total of 60 EI and 60 EF pots with plants of similar size were selected and pruned to a height of 10 cm from the soil substrate surface. Subsequently, three soil moisture conditions were established: 15% (low moisture), 30% (control moisture), and 60% (high moisture), each soil moisture condition with 20 EI and 20 EF plants. Plants were maintained at these soil moisture conditions for 4 weeks. During the trial, each pot was weighed every evening at 18:00 h. The moisture content of each pot was adjusted on a daily basis. After 4 weeks, 18 EI and 18 EF plants per soil moisture treatment were selected, and subsequently, six plants were randomly chosen and grouped together to generate single experimental replicates (three replicates per combination of treatments) for measurements. All leaf blades from each plant were harvested and pooled together within their respective plant group in aluminum foils, frozen in liquid nitrogen, and stored at – 80 °C until subsequent determination of plant oxidative damage, antioxidant contents, and gene expression levels.

Determination of the Oxidative Damage and Antioxidant Contents

Distinct kits of enzyme-linked immunosorbent assays (ELISA) (FANKEL Industrial Co. Ltd., Shanghai, China) were employed to determine the contents of MDA (an indicator of oxidative damage), enzymatic antioxidants (i.e., CAT, POD, SOD), and non-enzymatic antioxidants (i.e., AsA, Car, GSH, Pro) [39, 40]. Following kit instructions, measurements were performed as follows: 0.5 g of freeze-dried leaf blade samples was ground, and 5 mL of phosphate-buffered saline solution (PBS, pH 7.4) was added and centrifuged at 750 (×g) for 20 min at 4 ℃ using a refrigerated centrifuge (H-1850R, Xiangyi®, Hunan, China). Aliquots of 10 µL of supernatants were placed within wells of microtiter plates and diluted in 40 µL of PBS solution that included equal volumes of MDA and antioxidant standards. Plates were incubated for 30 min at 37 °C and washed five times with washing solution to remove unbound substances. The 50 µL of chromogen solution A and the same volume of chromogen solution B were added and incubated for 15 min at 37 ℃ in the dark. A volume of 50 µL of termination solution was added to the wells, and the optical density (OD) value of each sample was measured at 450 nm using a microplate reader (DR6000, Hach, Loveland, CO, USA). The limit of detection (LOD) and limit of quantification (LOQ) for the compounds were 0.10 and 12 $\mu g \bullet g^{-1}$ for MDA, 0.30 and 9 $U \bullet g^{-1}$ for CAT, 0.0006 and 0.0028 $U \bullet g^{-1}$ for POD, 0.12 and 4.20 $U \bullet g^{-1}$ for SOD, 0.10 and 80 $\mu g \bullet g^{-1}$ for AsA, 1 and 200 $\mu g \bullet g^{-1}$ for Car, 1 and 480 $\mu g \bullet g^{-1}$ for GSH, and 0.001 and 0.02 $\mu g \bullet g^{-1}$ for Pro. Separated calibration curves were performed for the distinct compounds that were measured and each curve was generated from specific standards. The contents of MDA and antioxidants were calculated by comparing the OD values with their corresponding calibration curves.

RNA Extraction and Transcriptome Sequencing

RNA-seq was used to quantify the gene expression of plant transcriptomes. RNA was extracted from leaf blade samples (recall that there were three replicates per combination of

treatments). The extraction was performed on 3 μg of ground leaf blades using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The extracted RNA was cleaned with the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). The concentration and purity of the RNA were determined with a Qubit®2.0 fluorometer (Life Technologies, Inc., USA) and the RNA integrity number (RIN) with an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., USA). To construct the sequencing library, the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, San Diego, CA, USA) was utilized, and transcriptome sequencing was performed on the Illumina HiSeq-PE250 platform by Biomarker Technologies Company (Beijing, China). The sequencing depth allowed to capture plant RNAs.

For comparing the expression of plant genes, EF and 30% soil moisture samples were used as controls while EI, 15%, and 60% samples were taken as the experimental groups. We performed gene expression comparisons to test the single effects of the endophyte status (EI = EI-30% vs. EF-30%), low soil moisture (15% = EF-15% vs. EF-30%), and high soil moisture (60% = EF-60% vs. EF-30%) and combined effects of the endophyte & low soil moisture (EI & 15% = EI-15% vs. EF-30%) and endophyte & high soil moisture (EI & 60% = EI-60% vs. EF-30%). The gene expression comparisons in EI and EF plants at different soil moisture conditions are shown in Table S1. A total of 138.42-Gb clean reads were obtained from sequencing samples, with around 6.34-Gb clean reads per sample and above 89.19% of Q30 bases. There were 92,964 unigenes (N50 of 1677 base pair (bp)), with a mean length of 865.18 bp and 60,242 transcripts longer than 1000 bp (24.54%). Genes were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) with the KEGG Orthology-Based Annotation System (KOBAS) software [41]. Genes were also annotated using the non-redundant protein sequences (NR) from the National Center for Biotechnology Information (NCBI), Protein family (Pfam), Clusters of Orthologous Groups of proteins (KOG/COG/eggNOG), a manually annotated and reviewed protein sequence database (Swiss-Prot), KEGG, and Gene Ontology (GO) databases. The annotated unigenes were 42,618. The expression level of each annotated gene was normalized based on transcript lengths and library size. The normalized gene expression levels were then calculated as fragments per kilobase per million mapped fragments (FPKM).

We investigated the expression of genes associated with antioxidant pathways in EI and EF plants at different soil moisture conditions. Specifically, we calculated and identified the differentially expressed gene (DEG)–related comparisons regarding the EI, 15%, 60%, EI & 15%, and EI & 60% effects. Plant genes associated with antioxidant pathways were identified according to KEGG_annotation, NR_annotation, Pfam_annotation, Swissprot_annotation, and GO_annotation. Our analysis was focused on plant genes associated with the enzymatic antioxidants POD and non-enzymatic antioxidants AsA, Car, GSH, and Pro pathways, as delineated in the KEGG pathways in https://www.genome.jp/kegg/ and BMK KEGG pathway data (see genes in Table S2). The expression of genes related to MDA, CAT, and SOD pathways was not affected by the EI, 15%, 60%, EI & 15%, and EI & 60% effects (see Table S3); therefore, these genes were not included in further analyses. The RNA-seq data used in the present study were deposited in the Sequence Read Archive (SRA) of the NCBI database under accession number PRJNA748183 and previously used for other studies (i.e., [20, 21]).

Statistical Analysis

The effects of the plant endophytic status and soil moisture treatments on the contents of oxidative damage indicator MDA; enzymatic antioxidants CAT, POD, and SOD; non-enzymatic antioxidants AsA, Car, GSH, and Pro were analyzed using two-way analysis of variance (two-way ANOVA) in the SPSS 22.0 program assuming normal distribution of errors (SPSS Inc., USA). The Duncan method was used for post hoc analyses to test the difference among means. All analyses met the ANOVA assumptions (residual independence, normality, and variance homogeneity). The results are presented as mean ± SEM (standard error of the mean) with a significance level of $P < 0.05$. Graphs were generated using the Origin 2018 software (OriginLab Corporation, USA). The DEGs were determined utilizing the exactTest function of edgeR package in the R software (version 3.5.0), with fold change (FC) > 1.5 and a false discovery rate (FDR) < 0.05 [42].

Results

Effects of Endophyte and Soil Moisture Treatments on Plant Oxidative Levels

The effect of the endophyte on the oxidative damage indicator MDA depended on the soil moisture conditions (i.e., an endophyte × soil moisture interaction was observed) (Fig. 1).

At 30% soil moisture (control), the endophyte reduced MDA contents of plants. The MDA contents of plants were higher in EF-15% than EF-30%, but not different between EF-60% and EF-30%. The MDA contents did not differ between EI-15% and EF-30% but were lower in EI-60% than EF-30%. The endophyte reduced the MDA contents in 15% soil moisture conditions (Fig. 1).

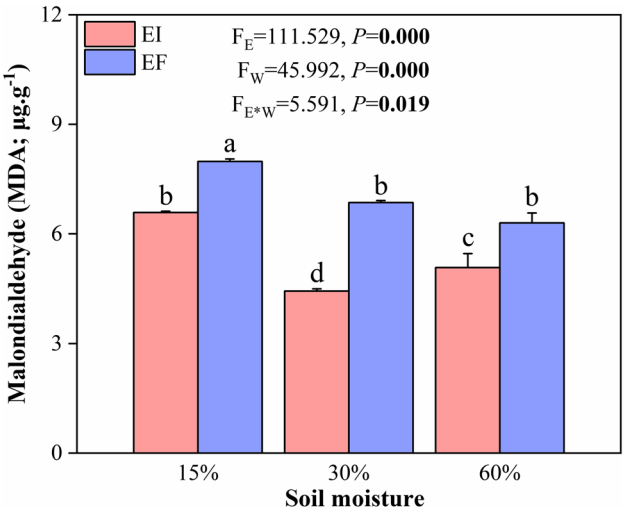


Fig. 1 Malondialdehyde content in leaf blades of 3-month-old *Achnatherum inebrians* plants infected (EI) with or free (EF) of *Epichloë gansuensis* endophytes under 15%, 30%, and 60% soil moisture conditions. Each panel contains *F* and *P* values associated with the ANOVA factors plant endophyte status (*E*), soil moisture (*M*), and $E \times M$ interaction. Values in bold are significant at $P < 0.05$. Bar are means ± SEM ($n = 3$)

Effects of Endophyte and Soil Moisture Treatments on Plant Enzymatic Antioxidant Contents

The effect of the endophyte on contents of CAT, POD, and SOD depended on the soil moisture conditions (i.e., an endophyte × soil moisture interaction was observed) (Fig. 2).

At 30% of soil moisture (control), the endophyte reduced the CAT contents. The CAT contents were not different between EF-15% and EF at 30%, but higher in EF-60% than EF-30%. The CAT contents were higher in both EI-15% and EI-60% than EF-30%. The endophyte increased CAT contents at 15% soil moisture (Fig. 2a).

At 30% of soil moisture (control), the endophyte reduced the POD contents. The POD contents were lower in both EF-15% and EF-60% than EF-30%. Similarly, the POD contents were also lower in EI-15% and EI-60% than EF-30%. The endophyte increased POD contents at 15% soil moisture (Fig. 2b).

At 30% soil moisture (control), the endophyte reduced the SOD contents. The SOD contents were lower in EF-15% than EF-30% but higher in EF-60% than EF-30%. The SOD contents were higher in EI-15% than EF-30% but not different between EI-60% and EF- 30%. The endophyte increased SOD contents in 15% soil moisture (Fig. 2c).

Effects of Endophyte and Soil Moisture Treatments on Plant Non-enzymatic Antioxidant Contents

The effect of the endophyte on the contents of AsA, Car, GSH, and Pro depended on the soil moisture conditions (i.e., an endophyte × soil moisture interaction was observed) (Fig. 3).

At 30% soil moisture (control), the endophyte increased the AsA contents. The AsA contents were lower in EF-15% than EF-30% but not different between EF-60% and EF-30%. The AsA contents were higher in EI-15% than EF-30% but

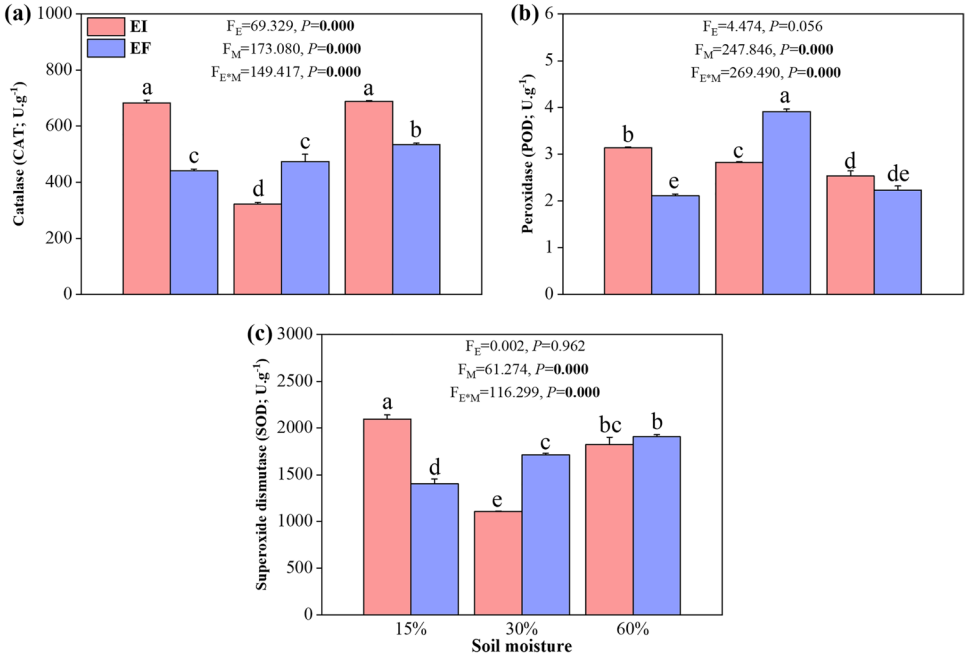


Fig. 2 Contents of enzymatic antioxidants catalase (**a**), peroxidase (**b**), and superoxide dismutase (**c**) in leaf blades of 3-month-old *Achnatherum inebrians* plants infected with (EI) or free (EF) of *Epichloë gansuensis* endophyte under 15%, 30%, and 60% soil moisture conditions. Enzyme contents are expressed as units (U) per gram (g) of freeze-dried leaf samples. Each panel contains *F* and *P* values associated with the ANOVA factors plant endophyte status (*E*), soil moisture (*M*), and $E \times M$ interaction. Values in bold are significant at $P < 0.05$. Bar are means ± SEM ($n = 3$)

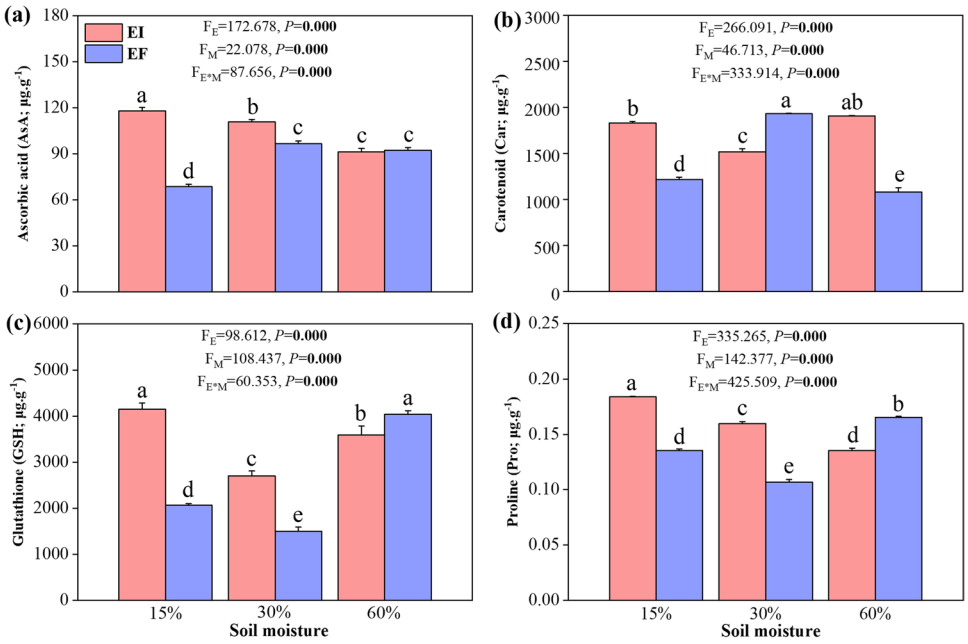


Fig. 3 Contents of non-enzymatic antioxidants ascorbic acid (**a**), carotenoid (**b**), glutathione (**c**), and proline (**d**) in leaf blades of 3-month-old *Achnatherum inebrians* plants infected with (EI) or free (EF) of *Epichloë gansuensis* endophyte under 15%, 30%, and 60% soil moisture conditions. Contents of non-enzymatic antioxidants are expressed as micrograms per gram (g) of freeze-dried leaf samples. Each panel contains *F* and *P* values associated with the ANOVA factors plant endophyte status (*E*), soil moisture (*M*), and *E* × *M* interaction. Values in bold are significant at $P < 0.05$. Bar are means ± SEM ($n = 3$)

not different between EI-60% and EF-30%. The endophyte increased the AsA contents at 15% soil moisture (Fig. 3a).

At 30% soil moisture (control), the endophyte reduced the Car contents. The Car contents were lower in both EF-15% and EF-60% than EF-30%. The Car contents were lower in EI-15% than EF-30% but not different between EI-60% and EF-30%. The endophyte increased the Car contents at 15% soil moisture (Fig. 3b).

At 30% soil moisture (control), the endophyte increased the GSH contents. The GSH contents were higher at both EF-15% and EF-60% than EF-30%. Similarly, the GSH contents were also higher in EI-15% and EI-60% than EF-30%. The endophyte increased the GSH contents at 15% soil moisture (Fig. 3c).

At 30% soil moisture (control), the endophyte increased the Pro contents. The Pro contents were higher in both EF-15% and EF-60% than EF-30%. Similarly, the Pro contents were also higher in EI-15% and EI-60% than EF-30%. The endophyte increased the Pro contents at 15% soil moisture (Fig. 3d).

Effects of Endophyte and Soil Moisture Treatments on Plant Genes Associated with Antioxidant Pathways

We found 54 DEGs associated with antioxidant pathways in plant leaves, including 23 POD-, 2 AsA-, 3 Car-, 20 GSH-, and 6 Pro-related genes (Fig. 4, Table S2). These DEGs were primarily enriched in the pathways "Phenylpropanoid biosynthesis," "Ascorbate and aldarate metabolism," "Carotenoid biosynthesis," "Glutathione metabolism," and "Arginine and proline metabolism" (Table S2).

In agreement with the endophyte-mediated reduction in POD contents in 30% soil moisture, the presence of endophyte reduced the expression of most of the plant POD genes detected (i.e., EI condition). In contrast with the soil moisture–based reduction in POD contents, the expressions of most plant POD genes were upregulated at 15% and 60% soil moisture conditions. In line with the endophyte/soil moisture–mediated reduction in POD contents, the plant POD genes that were downregulated in both EI & 15% and EI & 60% treatments showed higher changes in expression levels than the POD genes that were upregulated (Fig. 4a).

Consistent with the endophyte-mediated increase in AsA contents in 30% soil moisture, the endophyte increased the expression of plant *MIOX* and *F3H* genes that code for AsA biosynthetic enzymes (i.e., EI condition) [43]. Despite the reduction in AsA contents in 15% soil moisture, the expression of plant genes associated with AsA pathway were not different in this condition. In line with the unaltered AsA contents in high soil moisture, the expression of the plant AsA-derived genes did not change in the 60% condition. In line with the endophyte/soil moisture–mediated effects on AsA contents, the combined EI & 15% treatments increased the expression of the AsA biosynthesis gene *F3H* while the combined EI & 60% treatments did not alter the expression of plant AsA-derived genes (Fig. 4b).

In contrast with the endophyte-mediated reduction in Car contents in 30% soil moisture, the endophyte increased the expression of the plant *crtB* gene that codes for a Car biosynthesis enzyme. Similarly, against expectations based on the soil moisture–based reduction in Car contents, the expression of the Car-metabolizing plant *LUT1* gene was downregulated in the 15% condition while unchanged in

the detected plant Car-derived genes in the 60% condition. Contrary to the endophyte/soil moisture–mediated effects on Car contents, both EI & 15% and EI & 60% treatments increased the expression of the Car biosynthesis plant genes *crtB* and *LcyE* (Fig. 4c).

Consistent with the endophyte-mediated increase in GSH contents in 30% soil moisture, the endophyte reduced the expression of most plant GST genes that code for enzymes that metabolize GSH (i.e., EI condition). In line with soil moisture–based increase in GSH contents, both 15% and 60% soil moisture conditions also reduced the expression of GSH-metabolizing plant GST genes. In line with the endophyte/soil moisture–mediated increase in GSH contents, the combined EI & 15% treatments simultaneously increased the expression of plant *gpx* and *gshB* genes encoding GST biosynthetic enzymes and reduced the expression of GSH-metabolizing GST genes. Similarly, the combined EI & 60% treatments reduced the expression of GSH-metabolizing GST genes (Fig. 4d).

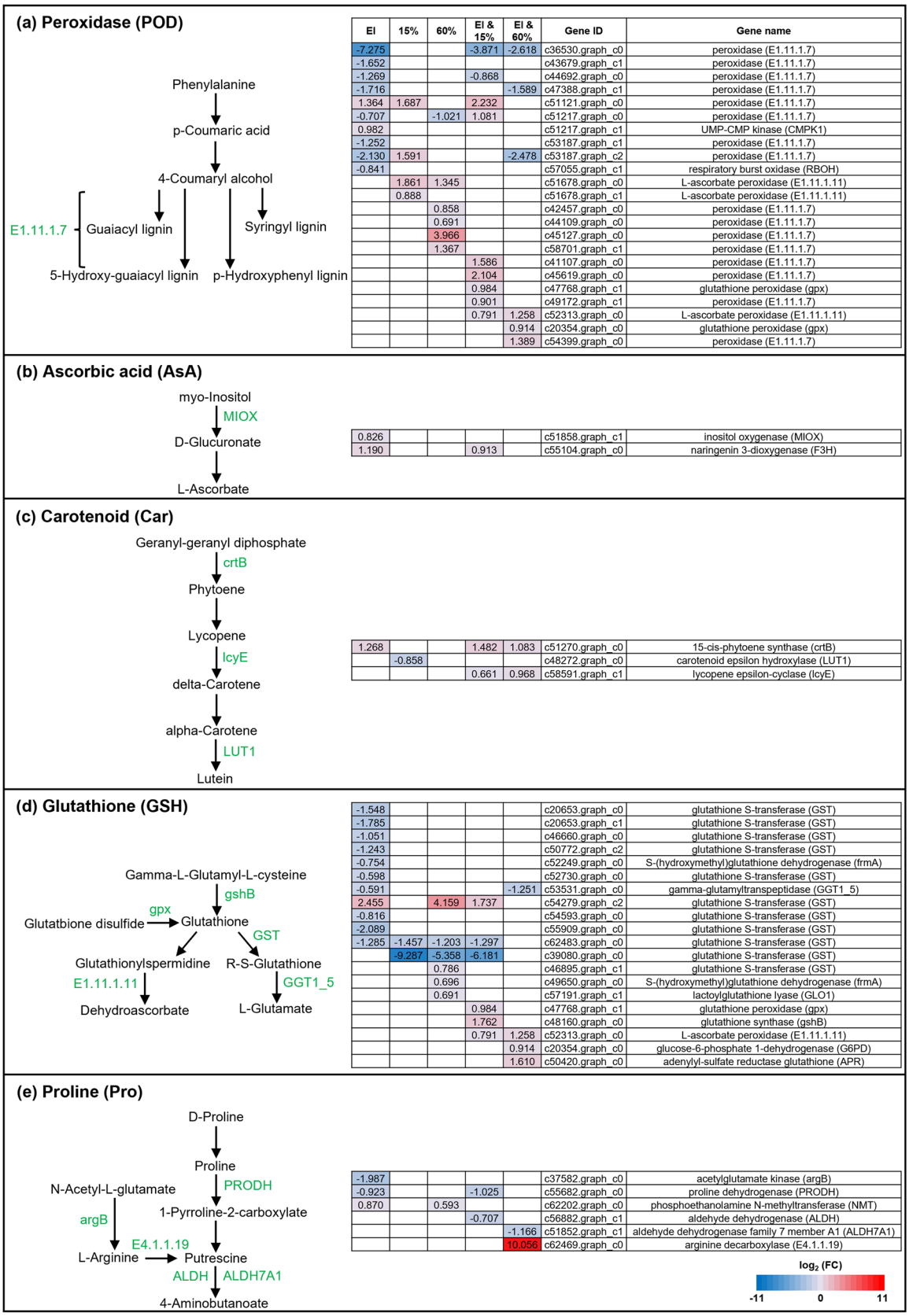
In agreement with the endophyte-mediated increase in Pro contents in 30% soil moisture, the endophyte reduced the expression of the plant gene *PRODH* that codes for an enzyme that metabolizes Pro (i.e., EI condition). Paradoxically, considering the low soil moisture–based increase in Pro contents, the detected plant genes associated with Pro pathway were not differentially expressed in the 15% condition. In line with the high soil moisture–based increase in Pro contents, the plant *NMT* gene that codes for a Pro biosynthesis enzyme was upregulated in the 60% condition [44]. In line with the endophyte/soil moisture–mediated increase in Pro contents, EI & 15% and EI & 60% treatments reduced the expression of plant *PRODH*, *ALDH*, and *ALDH7A1* genes that code for enzymes that metabolize Pro (Fig. 4e).

Discussion

We investigated the effects of the endophyte *E. gansuensis* and distinct soil moisture conditions on the level of oxidative damage, contents of antioxidants, and expression of plant genes related to antioxidant pathways in *A. inebrians* plants. As expected, the oxidative damage in EF plants, quantified via MDA contents, was elevated at low soil moisture but unaltered at high soil moisture. We hypothesized that the endophyte presence would reduce the plant oxidative damage specially at low soil moisture and our results agreed with this. The elevated oxidative damage in EF plants at low soil moisture was associated with a general reduction in antioxidant contents. We also expected that the endophyte would increase the antioxidant contents in plants specially at low soil moisture and the observed contents of both enzymatic and non-enzymatic antioxidants confirmed this hypothesis. The endophyte also increased the contents of most non-enzymatic antioxidants in plants grown at control soil moisture which may have contributed to the reduction in the oxidative damage. We predicted that the endophyte effects on the expression of plant genes associated with antioxidant pathways would be aligned with the changes in the patterns of accumulation/reduction of antioxidants across soil moisture conditions and as expected, the endophyte-mediated increases in antioxidant contents were generally associated with changes in the expression of plant genes that contributed to the accumulation of antioxidants in plant tissues.

Abiotic stresses generally trigger oxidative damage in plants [45]. Our results showed that the association of plants with *Epichloë* endophytes reduced the oxidative damage caused by the water restriction by 17.55%. *Epichloë*-mediated reductions in oxidative damage have also been documented in *Roegneria kamoji*, *Lolium perenne*, and *Festuca sinensis* plants subjected to water deficit [46–48]. In addition to drought, the presence of *Epichloë* endophytes in plants also alleviates the oxidative damage caused by other environmental stressors. For instance, the endophyte *E. gansuensis* reduced the MDA contents of plants grown in heavy metal–contaminated soils or exposed to fungal phytopathogens [31, 49]. Low MDA contents were also observed in *Hordeum brevisubulatum–Epichloë bromicola* plants grown in soils with elevated salinity [50].

Epichloë endophytes increased the contents of most antioxidants in plants grown under low soil moisture. Similar results have been documented in other plant-endophyte associations. *Epichloë* endophytes enhanced the contents of APX, CAT, and GR in *Festuca arizonica* and *Achnatherum robustum* plants subjected to drought stress [29]. The *Epichloë* presence in drought-treated *E. dahuricus* plants increased the contents of the antioxidants APX, CAT, POD, SOD, and Pro [36]. Antioxidants CAT, POD, and polyphenol oxidase (PPO) were also higher in endophyte-infected compared to endophyte-free *L. perenne* plants that were grown under water deficit [51]. In agreement with our hypothesis, the *Epichloë*-mediated enhancement in plant antioxidant contents at low soil moisture was correlated with reduced oxidative damage [27]. This pattern of change in antioxidant/oxidative damage has been observed in several plant-*Epichloë* associations (e.g., [52–54]). For instance, increased contents of APX, CAT, POD, SOD, GR, and Pro and reduced levels of ROS were documented in endophyte-symbiotic *E. dahuricus*, *F. arizonica*, and *A. robustum* plants grown at low soil moisture [29, 33]. It is expected that the combined effect of endophytes increasing antioxidant contents and reducing oxidative damage substantially enhance the fitness of their plant hosts especially under stress situations (e.g., [55, 56]). In fact, previous published findings have shown a higher performance of *A. inebrians* plants infected with *E. gansuensis* endophytes compared



EI	15%	60%	EI & 15%	EI & 60%	Gene ID	Gene name
-7.275			-3.871	-2.618	c36530.graph_c0	peroxidase (E1.11.1.7)
-1.652					c43679.graph_c1	peroxidase (E1.11.1.7)
-1.269			-0.868		c44692.graph_c0	peroxidase (E1.11.1.7)
-1.716				-1.589	c47388.graph_c1	peroxidase (E1.11.1.7)
1.364	1.687		2.232		c51121.graph_c0	peroxidase (E1.11.1.7)
-0.707		-1.021	1.081		c51217.graph_c0	peroxidase (E1.11.1.7)
0.982					c51217.graph_c1	UMP-CMP kinase (CMPK1)
-1.252					c53187.graph_c1	peroxidase (E1.11.1.7)
-2.130	1.591			-2.478	c53187.graph_c2	peroxidase (E1.11.1.7)
-0.841					c57055.graph_c1	respiratory burst oxidase (RBOH)
	1.861	1.345			c51678.graph_c0	L-ascorbate peroxidase (E1.11.1.11)
	0.888				c51678.graph_c1	L-ascorbate peroxidase (E1.11.1.11)
		0.858			c42457.graph_c0	peroxidase (E1.11.1.7)
		0.691			c44109.graph_c0	peroxidase (E1.11.1.7)
		3.966			c45127.graph_c0	peroxidase (E1.11.1.7)
		1.367			c58701.graph_c1	peroxidase (E1.11.1.7)
			1.586		c41107.graph_c0	peroxidase (E1.11.1.7)
			2.104		c45619.graph_c0	peroxidase (E1.11.1.7)
			0.984		c47768.graph_c1	glutathione peroxidase (gpx)
			0.901		c49172.graph_c1	peroxidase (E1.11.1.7)
			0.791	1.258	c52313.graph_c0	L-ascorbate peroxidase (E1.11.1.11)
				0.914	c20354.graph_c0	glutathione peroxidase (gpx)
				1.389	c54399.graph_c0	peroxidase (E1.11.1.7)

0.826					c51858.graph_c1	inositol oxygenase (MIOX)
1.190			0.913		c55104.graph_c0	naringenin 3-dioxygenase (F3H)

1.268			1.482	1.083	c51270.graph_c0	15-cis-phytoene synthase (crtB)
	-0.858				c48272.graph_c0	carotenoid epsilon hydroxylase (LUT1)
			0.661	0.968	c58591.graph_c1	lycopene epsilon-cyclase (lcyE)

-1.548					c20653.graph_c0	glutathione S-transferase (GST)
-1.785					c20653.graph_c1	glutathione S-transferase (GST)
-1.051					c46660.graph_c0	glutathione S-transferase (GST)
-1.243					c50772.graph_c2	glutathione S-transferase (GST)
-0.754					c52249.graph_c0	S-(hydroxymethyl)glutathione dehydrogenase (frmA)
-0.598					c52730.graph_c0	glutathione S-transferase (GST)
-0.591				-1.251	c53531.graph_c0	gamma-glutamyltranspeptidase (GGT1_5)
2.455		4.159	1.737		c54279.graph_c2	glutathione S-transferase (GST)
-0.816					c54593.graph_c0	glutathione S-transferase (GST)
-2.089					c55909.graph_c0	glutathione S-transferase (GST)
-1.285	-1.457	-1.203	-1.297		c62483.graph_c0	glutathione S-transferase (GST)
	-9.287	-5.358	-6.181		c39080.graph_c0	glutathione S-transferase (GST)
		0.786			c46895.graph_c1	glutathione S-transferase (GST)
		0.696			c49650.graph_c0	S-(hydroxymethyl)glutathione dehydrogenase (frmA)
		0.691			c57191.graph_c1	lactoylglutathione lyase (GLO1)
			0.984		c47768.graph_c1	glutathione peroxidase (gpx)
			1.762		c48160.graph_c0	glutathione synthase (gshB)
			0.791	1.258	c52313.graph_c0	L-ascorbate peroxidase (E1.11.1.11)
				0.914	c20354.graph_c0	glucose-6-phosphate 1-dehydrogenase (G6PD)
				1.610	c50420.graph_c0	adenylyl-sulfate reductase glutathione (APR)

-1.987					c37582.graph_c0	acetylglutamate kinase (argB)
-0.923			-1.025		c55682.graph_c0	proline dehydrogenase (PRODH)
0.870		0.593			c62202.graph_c0	phosphoethanolamine N-methyltransferase (NMT)
			-0.707		c56882.graph_c1	aldehyde dehydrogenase (ALDH)
				-1.166	c51852.graph_c1	aldehyde dehydrogenase family 7 member A1 (ALDH7A1)
				10.056	c62469.graph_c0	arginine decarboxylase (E4.1.1.19)

◂**Fig. 4** Changes in the expression levels of plant genes associated with antioxidant pathways associated with peroxidase (**a**), ascorbic acid (**b**), carotenoid (**c**), glutathione (**d**), and proline (**e**) in leaves of *Achnatherum inebrians* plants. The single effects of *Epichloë gansuensis* endophyte infection (EI), low soil moisture (15%), high soil moisture (60%), and the combined effects of *E. gansuensis* infection & low soil moisture (EI & 15%) and *E. gansuensis* infection & high soil moisture (EI & 60%) are shown. Simplified antioxidant pathways are depicted at the left, and differentially expressed genes (DEGs) are highlighted in green. Red and blue colors indicate significant differences in gene expression levels expressed as $\log_2$ fold change (FC) ($n = 3$)

to endophyte-free plants when subjected to water deficit [17, 19, 20]. It is worth mentioning that *E. gansuensis* also increased the contents of many antioxidants of *A. inebrians* plants under the control soil moisture condition, and these enhanced antioxidant contents may have also contributed to alleviate the oxidative damage caused by the water deficit treatment [48, 57].

Although the endophyte-mediated enhancement in plant antioxidants has been extensively documented [58–60], the mechanisms by which endophytes alter antioxidant contents in plants are not entirely clear. Our results suggest that at least part of the *Epichloë*-mediated increase in antioxidant contents in plants was caused by the endophyte stimulation of the host production of antioxidants or the endophyte stabilization of host antioxidants. Regulation of the plant antioxidant production by endophytes has been previously reported. Increased GSH contents in cadmium-treated *Sedum alfredii* plants associated with *Sphingomonas* sp. strain SaMR12 were apparently caused by the positive effects of the bacterium on the upregulated expression of plant *GS* and *GSH1* genes that coded for antioxidant biosynthetic enzymes [61]. The arbuscular mycorrhizal fungus *Funneliformis mosseae* increased APX, CAT, and GR antioxidant contents in *Citrullus lanatus* plants grown in contrasting salinity-alkalinity conditions via upregulating the expression of plant genes associated with the biosynthesis of these antioxidants [36]. Similarly, the presence of the endophyte *Piriformospora indica* in *Citrus sinensis* plants increased the expression of plant genes *CsPOD* and *CsCAT1* that coded for biosynthetic enzymes of CAT and POD antioxidants [62]. It is worth mentioning that although most of the plant gene expression results were aligned with the reported antioxidant contents, there was a lack of alignment in the findings associated with the Car. This may be because the biosynthesis of Car is complex, involving multiple enzymes and reaction steps, therefore it is likely that the expressions of the few genes reported in the present study were not sufficient to influence the total content of the antioxidant [63, 64].

Conclusion

Our findings provide strong circumstantial evidence that the drought-derived oxidative damage in *A. inebrians* plants was probably alleviated by ability of *Epichloë* endophytes to increase antioxidant contents in plants and upregulate plant genes related to antioxidant pathways. The endophyte-based alleviation of the plant oxidative damage may be associated with the endophyte's capacity to provide antioxidants, promotes the host production of antioxidants, or stimulates the activities of the existing host antioxidants [7, 31, 57, 65, 66]. Plant gene expression results suggest that at least part of the endophyte-based reduction in oxidative damage would be associated with the fungal regulation of plant antioxidants. Furthermore, studies would be warranted to determine the relative contribution of the *Epichloë*-derived antioxidants in the alleviation of the plant oxidative damage and if endophytes can alter kinetic of reaction of plant antioxidants. Our findings provide indicative evidence regarding the endophytic regulation of antioxidants in plants and suggest novel avenues for future research to understand how plant-endophyte associations respond to environmental stresses.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-024-02391-2.

Acknowledgements We would like to thank Cory Matthew for the critical revision of earlier versions of the manuscript.

Author Contribution X.M.N, Z.R.Z, and X.X.Z designed the experiment. X.M.N and Z.R.Z. performed the experiment and collected the data. X.M.N and D.A.B analyzed the data. X.M.N, X.X.Z, and D.A.B wrote the manuscript. Z.B.N and C.J.L provide resources. All authors reviewed the manuscript and agreed with the final version.

Funding This work was financially supported by the National Nature Science Foundation of China (31772665), the Fundamental Research Funds for the Central Universities (jbky-2022-ey21), and Lanzhou University, and Gansu Provincial Science and Technology Major Projects (23ZDNA009). D.A.B acknowledges the research support provided by the Strategic Science Investment Fund (SSIF) from the New Zealand Ministry of Business, Innovation and Employment (MBIE), and the Catalyst: Leaders New Zealand-China Scientist Exchange program from the Royal Society of New Zealand (agreement number: CHN-AGR2301).

Data Availability The biochemical data measured in the current study are available from the corresponding author on reasonable request. The RNA-seq data is deposited in the Sequence Read Archive (SRA) of the NCBI database under accession number PRJNA748183.

Declarations

Competing Interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Gupta A, Rico-Medina A, Caño-Delgado AI (2020) The physiology of plant responses to drought. Science 368:266–269. https://doi.org/10.1126/science.aaz7614
2. Vicente-Serrano SM, Quiring SM, Pena-Gallardo M, Yuan SS, Dominguez-Castro F (2020) A review of environmental droughts: increased risk under global warming? Earth-Sci Rev 201:102953. https://doi.org/10.1016/j.earscirev.2019.102953
3. Bastias DA, Martínez-Ghersa MA, Ballaré CL, Gundel PE (2017) *Epichloë* fungal endophytes and plant defenses: not just alkaloids. Trends Plant Sci 22:939–948. https://doi.org/10.1016/j.tplants.2017.08.005
4. Bennett AE, Groten K (2022) The costs and benefits of plant–arbuscular mycorrhizal fungal interactions. Annu Rev Plant Biol 73:649–672. https://doi.org/10.1146/annurev-arplant-102820-124504
5. Anand U, Pal T, Yadav N, Singh VK, Tripathi V, Choudhary KK, Shukla AK, Sunita K, Kumar A, Bontempi E, Ma Y, Kolton M, Singh AK (2023) Current scenario and future prospects of endophytic microbes: promising candidates for abiotic and biotic stress management for agricultural and environmental sustainability. Microb Ecol 86:1455–1486. https://doi.org/10.1007/s00248-023-02190-1
6. Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol 55:315–340. https://doi.org/10.1146/annurev.arplant.55.031903.141735
7. Chen L, Li XZ, Li CJ, Swoboda GA, Young CA, Sugawara K, Leuchtmann A, Schardl CL (2015) Two distinct *Epichloë* species symbiotic with *Achnatherum inebrians*, drunken horse grass. Mycologia 107:863–873. https://doi.org/10.3852/15-019
8. Dastogeer KM (2018) Influence of fungal endophytes on plant physiology is more pronounced under stress than well-watered conditions: a meta-analysis. Planta 248:1403–1416. https://doi.org/10.1007/s00425-018-2982-y
9. Bastías DA, Gundel PE (2023) Plant stress responses compromise mutualisms with *Epichloë* endophytes. J Exp Bot 74:19–23. https://doi.org/10.1093/jxb/erac428
10. Li CJ, Nan ZB, Paul VH, Dapprich PD, Liu Y (2004) A new *Neotyphodium* species symbiotic with drunken horse grass (*Achnatherum inebrians*) in China. Mycotaxon 90:141-147. https://orcid.org/0000-0002-3287-2140
11. Leuchtmann A, Bacon CW, Schardl CL, White JF, Tadych M (2014) Nomenclatural realignment of *Neotyphodium* species with genus *Epichloë*. Mycologia 106:202–215. https://doi.org/10.3852/13-251
12. Johnson LJ, Bastías DA, Caradus JR, Chettri P, Forester NT, Mace WJ, Miller TA, Moon CD, Voisey CR, Zhang W, Card SD (2021). The dynamic mechanisms underpinning symbiotic *Epichloë*–grass interactions: implications for sustainable and resilient agriculture. Microbiome stimulants for crops. Woodhead Publishing. Pp. 73-108. https://doi.org/10.1016/B978-0-12-822122-8.00008-X
13. Card SD, Bastías DA, Zhang W, Hume DE, Caradus JR (2024) *Epichloë*–a key element of New Zealand's agricultural landscape. New Zeal J Bot 1-29. https://doi.org/10.1080/0028825X.2024.2331570
14. Li CJ, Gao JH, Nan ZB (2007) Interactions of *Neotyphodium gansuense*, *Achnatherum inebrians*, and plant-pathogenic fungi. Mycol Res 111:1220–1227. https://doi.org/10.1016/j.mycres.2007.08.012
15. Cheng C, Wang JF, Hou WP, Malik K, Zhao CZ, Niu XL, Liu YL, Huang R, Li CJ, Nan ZB (2021) Elucidating the molecular mechanisms by which seed-borne endophytic fungi, *Epichloë gansuensis*, increases the tolerance of *Achnatherum inebrians* to NaCl stress. Int J Mol Sci 22(24):13191. https://doi.org/10.3390/ijms222413191
16. Jin J, Wang C, Liu RG, Gong JY, Wang JF, Niu XL, Zheng R, Tang ZL, Malik K, Li CJ (2023) Soil microbial community compositions and metabolite profiles of *Achnatherum inebrians* affect phytoremediation potential in Cd contaminated soil. J Hazard Mater 459:132280. https://doi.org/10.1016/j.jhazmat.2023.132280
17. Xia C, Christensen MJ, Zhang XX, Nan ZB (2018) Effect of *Epichloë gansuensis* endophyte and transgenerational effects on the water use efficiency, nutrient and biomass accumulation of *Achnatherum inebrians* under soil water deficit. Plant Soil 424:555–571. https://doi.org/10.1007/s11104-018-3561-5
18. Zhong R, Bastías DA, Zhang XX, Li CJ, Nan ZB (2022) Vertically transmitted *Epichloë* systemic endophyte enhances drought tolerance of *Achnatherum inebrians* host plants through promoting photosynthesis and biomass accumulation. J Fungi 8(5):512. https://doi.org/10.3390/jof8050512
19. Xia C, Li NN, Zhang XX, Feng Y, Christensen MJ, Nan ZB (2016) An *Epichloë* endophyte improves photosynthetic ability and dry matter production of its host *Achnatherum inebrians* infected by *Blumeria graminis* under various soil water conditions. Fungal Ecol 22:26–34. https://doi.org/10.1016/j.funeco.2016.04.002
20. Zhao ZR, Kou MZ, Zhong R, Xia C, Christensen MJ, Zhang XX (2021) Transcriptome analysis revealed plant hormone biosynthesis and response pathway modification by *Achnatherum inebrians* under different soil moisture availability. J Fungi 7:640. https://doi.org/10.3390/jof7080640
21. Zhao ZR, Tian M, Zeng P, Christensen MJ, Kou MZ, Nan ZB, Zhang XX (2022) Modification of cuticular wax composition and biosynthesis by *Epichloë gansuensis* in *Achnatherum inebrians* at different growing periods. Agriculture 12:1154. https://doi.org/10.3390/agriculture12081154
22. Mittler R, Zandalinas SI, Fichman Y, Van Breusegem F (2022) Reactive oxygen species signalling in plant stress responses. Nat Rev Mol Cell Biol 23:663–679. https://doi.org/10.1038/s41580-022-00499-2
23. Juan CA, de la Lastra JMP, Plou FJ, Perez-Lebena E (2021) The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. Int J Mol Sci 22:4642. https://doi.org/10.3390/ijms22094642
24. Begum N, Wang L, Ahmad H, Akhtar K, Roy R, Khan MI, Zhao TJ (2022) Co-inoculation of arbuscular mycorrhizal fungi and the plant growth-promoting rhizobacteria improve growth and photosynthesis in tobacco under drought stress by up-regulating antioxidant and mineral nutrition metabolism. Microb Ecol 83:971–988. https://doi.org/10.1007/s00248-021-01815-7

25. Bamary Z, Einali A (2022) Changes in carbon partitioning and pattern of antioxidant enzyme activity induced by arginine treatment in the green microalga dunaliella salina under long-term salinity. Microb Ecol 84:1–15. https://doi.org/10.1007/s00248-021-01843-3
26. Kapoor D, Singh S, Kumar V, Romero R, Prasad R, Singh J (2019) Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plant Gene 19:100182. https://doi.org/10.1016/j.plgene.2019.100182
27. Hamilton CE, Gundel PE, Helander M, Saikkonen K (2012) Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. Fungal Divers 54:1–10. https://doi.org/10.1007/s13225-012-0158-9
28. Bastías DA, Ueno AC, Gundel PE (2023) Global change factors influence plant-*Epichloë* associations. J Fungi 9:446. https://doi.org/10.3390/jof9040446
29. Hamilton CE, Bauerle TL (2012) A new currency for mutualism? Fungal endophytes alter antioxidant activity in hosts responding to drought. Fungal Divers 54:39–49. https://doi.org/10.1007/s13225-012-0156-y
30. Nagabhyru P, Dinkins RD, Wood CL, Bacon CW, Schardl CL (2013) Tall fescue endophyte effects on tolerance to water-deficit stress. BMC Plant Biol 13:1–17. https://doi.org/10.1186/1471-2229-13-127
31. Zhang XX, Li CJ, Nan ZB (2010) Effects of cadmium stress on growth and antioxidative systems in *Achnatherum inebrians* symbiotic with *Neotyphodium gansuense*. J Hazard Mater 175:703–709. https://doi.org/10.1016/j.jhazmat.2009.10.066
32. Wang JF, Nan ZB, Christensen MJ, Li CJ (2018) Glucose-6-phosphate dehydrogenase plays a vital role in *Achnatherum inebrians* plants host to *Epichloë gansuensis* by improving growth under nitrogen deficiency. Plant Soil 430:37–48. https://doi.org/10.1007/s11104-018-3710-x
33. Zhang YP, Nan ZB (2007) Growth and antioxidative systems changes in *Elymus dahuricus* is affected by *Neotyphodium* endophyte under contrasting water availability. J Agron Crop Sci 193:377–386. https://doi.org/10.1111/j.1439-037X.2007.00279.x
34. Bindal S, Rana M, Srivastava D, Pavithra G (2020) Role of endophytic microbes against plant pathogens: a review. Asian J Plant Sci 19:54–62. https://doi.org/10.3923/ajps.2020.54.62
35. Sahu PK, Jayalakshmi K, Tilgam J, Gupta A, Nagaraju Y, Kumar A, Hamid S, Singh HV, Minkina T, Rajput VD (2022) ROS generated from biotic stress: effects on plants and alleviation by endophytic microbes. Front Plant Sci 13:1042936. https://doi.org/10.3389/fpls.2022.1042936
36. Li QS, Xie YC, Rahman MM, Hashem A, Abd Allah EF, Wu QS (2022) Arbuscular mycorrhizal fungi and endophytic fungi activate leaf antioxidant defense system of lane navel orange. J Fungi 8:282. https://doi.org/10.3390/jof8030282
37. He JD, Zou YN, Wu QS, Kuca K (2020) Mycorrhizas enhance drought tolerance of trifoliate orange by enhancing activities and gene expression of antioxidant enzymes. Sci Hortic 262:108745. https://doi.org/10.1016/j.scienta.2019.108745
38. Li NN, Zhao YF, Xia C, Zhong R, Zhang XX (2016) Effects of thiophanate-methyl on seed borne *Epichloë* fungal endophyte of *Achnatherum inebrians*. Pratacultural Sci 33(7):1306–1314. in Chinese with English abstract
39. Zhu YM, Wu QH, Lv HQ, Chen WX, Wang LZ, Shi SJ, Yang JG, Zhao PP, Li Yz, Christopher RS, Liu H, Feng RW (2020) Toxicity of different forms of antimony to rice plants: effects on reactive oxidative species production, antioxidative systems, and uptake of essential elements. Environ Pollut 263:114544. https://doi.org/10.1016/j.envpol.2020.114544
40. Wang B, Yang RC, Ji ZQ, Zhang HX, Zheng WB, Zhang HH, Feng FQ (2022) Evaluation of biochemical and physiological changes in sweet corn seeds under natural aging and artificial accelerated aging. Agron 12(5):1028. https://doi.org/10.3390/agronomy12051028
41. Xie C, Mao XZ, Huang JK, Ding Y, Wu JM, Dong S, Kong L, Gao G, Li CY, Wei LP (2011) KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res 39:316–322. https://doi.org/10.1093/nar/gkr483
42. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550. https://doi.org/10.1186/s13059-014-0550-8
43. Sun JH, Qiu C, Ding YQ, Wang Y, Sun LT, Fan K, Gai ZS, Dong GQ, Wang JG, Li XH, Song LB, Ding ZT (2020) Fulvic acid ameliorates drought stress-induced damage in tea plants by regulating the ascorbate metabolism and flavonoids biosynthesis. BMC genomics 21:1–13. https://doi.org/10.1186/s12864-020-06815-4
44. Tian LL, Zhao XL, Hu ZY, Liu J, Ma J, Fan YL, Liu DH (2023) iTRAQ-based proteomics identifies proteins associated with betaine accumulation in *Lycium barbarum* L. J Proteomics 290:105033. https://doi.org/10.1016/j.jprot.2023.105033
45. Choudhury FK, Rivero RM, Blumwald E, Mittler R (2017) Reactive oxygen species, abiotic stress and stress combination. Plant J 90(5):856–867. https://doi.org/10.1111/tpj.13299
46. Bu YY, Guo P, Ji YL, Zhang ST, Yu HS, Wang ZW (2019) Effects of *Epichloë sinica* on *Roegneria kamoji* seedling physiology under PEG-6000 simulated drought stress. Symbiosis 77:123–132. https://doi.org/10.1007/s13199-018-0570-3
47. Li F, Duan TY, Li YZ (2020a) Effects of the fungal endophyte *Epichloë festucae var lolii* on growth and physiological responses of perennial ryegrass cv. fairway to combined drought and pathogen stresses. Microorganisms 8:1917. https://doi.org/10.3390/microorganisms8121917
48. Xu WB, Li MM, Lin WH, Nan ZB, Tian P (2021) Effects of *Epichloë sinensis* endophyte and host ecotype on physiology of *Festuca sinensis* under different soil moisture conditions. Plants 10:1649. https://doi.org/10.3390/plans10081649
49. Zhang HJ, Li XJ, White JF, Wei XK, He YL, Li CJ (2021) *Epichloë* endophyte improves ergot disease resistance of host (*Achnatherum inebrians*) by regulating leaf senescence and photosynthetic capacity. J Plant Growth Regul 41:808–817. https://doi.org/10.1007/s00344-021-10340-3
50. Wang ZF, Li CJ, White J (2020) Effects of *Epichloë* endophyte infection on growth, physiological properties and seed germination of wild barley under saline conditions. J Agron Crop Sci 206:43–51. https://doi.org/10.1111/jac.12366
51. Li F, Deng J, Nzabanita C, Li YZ, Duan TY (2019) Growth and physiological responses of perennial ryegrass to an AMF and an *Epichloë* endophyte under different soil water contents. Symbiosis 79:151–161. https://doi.org/10.1007/s13199-019-00633-3
52. Ma MZ, Christensen MJ, Nan ZB (2015) Effects of the endophyte *Epichloë festucae var. lolii* of perennial ryegrass (*Lolium perenne*) on indicators of oxidative stress from pathogenic fungi during seed germination and seedling growth. Eur J Plant Pathol 141:571–583. https://doi.org/10.1007/s10658-014-0563-x
53. Chen TX, Johnson R, Chen SH, Lv H, Zhou JL, Li CJ (2018) Infection by the fungal endophyte *Epichloë* bromicola enhances the tolerance of wild barley (*Hordeum brevisubulatum*) to salt and alkali stresses. Plant Soil 428:353–370. https://doi.org/10.1007/s11104-018-3643-4
54. Amirikhah R, Etemadi N, Sabzalian MR, Nikbakht A, Eskandari A (2021) Gamma radiation negatively impacted seed germination, seedling growth and antioxidant enzymes activities in tall fescue infected with *Epichloë* endophyte. Ecotoxicol Environ Saf 216:112169. https://doi.org/10.1016/j.ecoenv.2021.112169
55. Wang JF, Hou WP, Christensen MJ, Li XZ, Xia C, Li CJ, Nan ZB (2020) Role of *Epichloë* endophytes in improving host

grass resistance ability and soil properties. J Agric Food Chem 68:6944–6955. https://doi.org/10.1021/acs.jafc.0c01396
56. Saedi T, Mosaddeghi MR, Sabzalian MR, Zarebanadkouki M (2021) Effect of *Epichloë* fungal endophyte symbiosis on tall fescue to cope with flooding-derived oxygen-limited conditions depends on the host genotype. Plant Soil 468:353–373. https://doi.org/10.1007/s11104-021-05105-6
57. Li XZ, Simpson WR, Song ML, Bao GS, Niu XL, Zhang ZH, Xu HF, Liu X, Li YL, Li CJ (2020) Effects of seed moisture content and *Epichloe* endophyte on germination and physiology of *Achnatherum inebrians*. S African J Bot 134:407–414. https://doi.org/10.1016/j.sajb.2020.03.022
58. Lee KD, Missaoui A, Mahmud K, Presley H, Lonnee M (2021) Interaction between grasses and *Epichloë* endophytes and its significance to biotic and abiotic stress tolerance and the rhizosphere. Microorganisms 9:2186. https://doi.org/10.3390/microorganisms9112186
59. Mosaddeghi MR, Hosseini F, Hajabbasi MA, Sabzalian MR, Sepehri M (2021) *Epichloë* spp. and *Serendipita indica* endophytic fungi: functions in plant-soil relations. Adv Agron 165:59–113. https://doi.org/10.1016/bs.agro.2020.09.001
60. Pan FS, Meng Q, Wang Q, Luo S, Chen B, Khan KY, Yang X, Feng Y (2016) Endophytic bacterium *Sphingomonas* SaMR12 promotes cadmium accumulation by increasing glutathione biosynthesis in *Sedum alfredii* Hance. Chemosphere 154:358–366. https://doi.org/10.1016/j.chemosphere.2016.03.120
61. Ye L, Zhao X, Bao EC, Cao K, Zou ZR (2019) Effects of arbuscular mycorrhizal fungi on watermelon growth, elemental uptake, antioxidant, and photosystem II activities and stress-response gene expressions under salinity-alkalinity stresses. Front Plant Sci 10:863. https://doi.org/10.3389/fpls.2019.00863
62. Pinto OHB, Costa FS, Rodrigues GR, da Costa RA, Fernandes GD, Pires OR, Barreto CC (2021) Soil Acidobacteria strain AB23 resistance to oxidative stress through production of carotenoids. Microb Ecol 81:169–179. https://doi.org/10.1007/s00248-020-01548-z
63. Sandmann G (2021) Diversity and origin of carotenoid biosynthesis: its history of coevolution towards plant photosynthesis. New Phytol 232:479–493. https://doi.org/10.1111/nph.17655
64. White JF Jr, Torres MS (2010) Is plant endophyte-mediated defensive mutualism the result of oxidative stress protection? Physiol Plant 138:440–446. https://doi.org/10.1111/j.1399-3054.2009.01332.x
65. Sadeghi F, Samsampour D, Seyahooei MA, Bagheri A, Soltani J (2020) Fungal endophytes alleviate drought-induced oxidative stress in mandarin (*Citrus reticulata* L.): toward regulating the ascorbate–glutathione cycle. Sci Hortic 261:108991 https://doi.org/10.1016/j.scienta.2019.108991
66. Chakrabarti M, Nagabhyru P, Schardl CL, Dinkins RD (2022) Differential gene expression in tall fescue tissues in response to water deficit. Plant Genome 15:e20199. https://doi.org/10.1002/tpg2.20199