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Epichloë Fungal Endophyte Symbiosis May Improve Tall Fescue Responses to Flooding and Oxygen-Limited Conditions

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Abstract

Background and aims There is little information about the effect of grass-fungal endophyte symbiota on plant performance under oxygen-limited conditions. This study aimed to investigate the effect of *Epichloë* endophyte symbiosis and tall fescue genotype on plant responses to oxygen stress in a greenhouse pot experiment.

Methods A greenhouse pot experiment was conducted with seven air-filled porosity levels in a sandy loam soil using two genotypes (75C and 75B) of tall fescue (*Festuca arundinacea* = *Schedonorus arundinaceus* Schreb.) infected with and without endophytic fungus *Epichlöe coenophiala* (E+ and E–, respectively). Some selected growth and physiological parameters were determined after nine-month application of the treatments.

Results The results showed that E+ plants benefited from endophytic symbiosis and showed slightly higher root and shoot development, more leaf chlorophyll, and lower catalase and ascorbate peroxidase activity than E– plants under poor aeration. The E– plants also coped with poor aeration conditions by forming adventitious roots at the soil surface, aerenchyma formation within the root tissue, and increased alcohol dehydrogenase (ADH) activity.

Conclusions The presence of endophyte improved the performance of the genotype E+ 75B under anaerobic conditions, while endophyte had an adverse effect on the performance of the genotype E+ 75C. In general, *Epichloë* endophyte presence decreased the flooding induced oxidative stress and prevented the formation and over-accumulation of reactive oxygen species in plant cells.

Introduction

Soil aeration, particularly at the vicinity of roots, is a vital soil physical property affecting plant growth. The ability of plants to grow and survive under limited soil aeration varies among different plant species. Although some plants such as rice are adapted to grow under limited soil aeration, most of the other plants are very sensitive to poor soil aeration. For instance, when the soil air-filled porosity (volume of air-filled pores divided by total porosity) drops below 10%, waterlogging stress (lack of oxygen and gas exchange) becomes a major limiting factor for plant growth and production (Grable and Siemer 1968; Busscher 1982).

Soil aeration is a dynamic soil property varying strongly with soil water content and bulk density (Busscher 1982). In waterlogging and near-saturated conditions, gas exchange between plants, microorganisms, and the soil becomes extremely limited as the oxygen diffusion coefficient in water is ca. 10,000 times lower than in the air (DeLaune and Reddy 2008). The deficiency of oxygen (hypoxia) or its absence (anoxia) results in various morphological, physiological, and anatomical changes in the plants that are harmful to plant growth and root respiration. The waterlogging stressed plants show symptoms such as reduced leaf and root biomass, leaf chlorosis and wilting, reduced shoot nitrogen content, and reduced leaf chlorophyll content (Pezeshki and DeLaune 2012; Zhao et al. 2014). In addition, oxygen deficiency causes the closure of stomata, reduction in CO_2 concentration in the leaves, and thus a decrease in photosynthesis (Luzhen et al. 2005; Else et al. 2008), and consequently, it might also increase the concentration of reactive oxygen species (ROS): superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), inside the plant cells. These oxygen species are all very reactive and cause severe damage to membranes, DNA and proteins, thus creating oxidative stress (Foyer et al. 1994).

Plants adapted to grow under poor aeration conditions incorporate varying morphological, anatomical, physiological, and molecular mechanisms (adaptive or protective) to cope with waterlogging stress. Adventitious root formation is an example of morphological adaptations (DeLaune and Reddy 2008; Justin and Armstrong 1991), while aerenchyma formation is the most common type of anatomical adaptation to waterlogging stress. Aerenchyma occurs when plant tissues containing enlarged gas spaces forming across the root, shoot, or leaf tissue of plants are involved in the storage, supply, and transport of atmospheric oxygen to root and their surrounding rhizospheric soil (Benz et al. 2007; Evans 2004). Switching to anaerobic fermentation through the use of the alcohol dehydrogenase enzyme (ADH) is a biochemical adaptation method in some plant species (Roberts et al. 1984; Dennis et al. 2000). In a study of ADH null mutants of maize (a maize mutant deficient in one of its ADH genes) under anoxic conditions, it was found that this mutant was more sensitive to anoxic than the wild-type plant (Johnson et al. 1989), indicating the importance of ADH in flooding tolerance of plants. Plants possess some low molecular mass antioxidants (e.g., ascorbate, glutathione, phenolic compounds, and tocopherols) and ROS scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) to control the level of ROS and to protect the cells under waterlogging conditions (Foyer et al. 1994; Yiu et al. 2011; Yordanova et al. 2004). For instance, the results of Yiu et al. (2011) suggested that the exogenous catechin markedly reduced the adverse effects of waterlogging in tomatoes by increasing the activity of CAT, APX and SOD and free radical scavenging system, and consequently reduction of H_2O_2 and O_2^- concentrations.

Besides anatomical, morphological and/or metabolic adaptation, plants may involve companions with microorganisms to overcome poor soil aeration conditions. The plant-endophyte association can be a good candidate. Fungal endophytes are defined as “fungi that complete their life cycle within the aerial portion of the host plants forming nonpathogenic, systemic, and usually intercellular associations” (Malinowski and Belesky 2000; Hume et al. 2016). Although it is shown that endophyte presence in the aboveground parts of cool-season grasses could improve soil organic carbon, rhizosphere microbial community, and consequently soil quality (Hosseini et al. 2015a,b) but less is known about their mechanistic contribution to plant performance under the oxygen-limited conditions (Adams et al. 2017; Arachevaleta et al. 1989; Song et al. 2015). The findings of Song et al. (2015) indicated that endophyte presence in *Hordeum brevisubulatum* was associated with greater root vitality, root biomass, and tiller production in the flooded conditions along with lower leaf wilting rate than endophyte-free plants. Whereas Arachevaleta et al. (1989) compared tillers of one genotype of tall fescue (*Festuca arundinacea* = *Schedonorus arundinaceus* Schreb.) infected with endophyte (E+) and free of endophyte (E–), but the endophyte symbiosis did not provide a benefit to plant growth under anoxic condition.

Tall fescue is an important perennial, cool-season bunchgrass up to 2 m tall hosting endophytic fungus *Epichlöe coenophiala* (Gibson and Newman 2001). The objective of this study was to investigate the effects of *Epichloë* endophyte infection on two selected genotypes (75B and 75C) of tall fescue under

oxygen-limited conditions. In particular, we aimed to address the following questions: Does the fungal endophyte improve plant performance under oxygen-limited conditions, and if so, what are the mechanisms? What are the mechanisms these two genotypes employ to cope with oxygen-limited conditions, and whether these mechanisms differ in the presence or absence of fungal endophyte? To do so, some growth, morphological, anatomical, metabolic, and physiological responses of two selected genotypes of tall fescue infected with or without endophyte (E + and E−, respectively) were compared under varying levels of oxygen supply in soil (soil air-filled porosity).

Material And Methods

Soil preparation and its physical and chemical characteristics

A sandy loam soil was collected from the top layer (0–30 cm) of a rangeland around Isfahan Province in Iran (32^0 46′ 42.6″ N and 52^0 44′ 33.7″ E). The collected soil was first air-dried and then passed through a sieve with a mesh size of 4 mm with minimal destruction of its aggregates. A subsample was selected and passed through a 2-mm sieve for the characterization of basic soil properties, including soil texture (determined by the pipette method, Gee and Bauder 1986), soil organic matter (SOM) content (determined by the wet-oxidation method, Walkley and Black 1934), and pH and electrical conductivity (EC) (determined in a saturated soil extract using a WTW pH-meter and an Elmerton EC-meter, respectively, Rhoades 1996; Thomas 1996). The soil texture was sandy loam, with 78.3 kg 100 kg^{-1} of sand (50–2000 µm) and 11.4 kg 100 kg^{-1} of clay (< 2 µm) contents. The soil was classified as non-saline (EC of saturated extract, 0.99 dS m^{-1}) and slightly alkaline (pH of saturated extract, 7.9) with low contents of organic matter (0.43 kg 100 kg^{-1}) and equivalent calcium carbonate (6.7 kg 100 kg^{-1}).

The soil water retention curve was also determined on the samples (< 4 mm with minimal destruction of aggregates) packed to a natural dry bulk density (BD) of 1.54 Mg m^{-3} in the stainless-steel cylindrical rings (5.2 cm diameter and 4.5 cm height). The soil water retention curve was measured at absolute matric potentials of 0, 1, 5, 7, 10, 20, 30, 40, 50, 60, 70, 100, 330, 500, 1000, 2000, 3000, 5000, 8000, 10000 and 15000 hPa using a combination of sandbox apparatus (Eijkelkamp, Giesbeek, The Netherlands) and pressure plate apparatus (Reynolds and Topp 2008). The studied soil had bi-modal pore size distribution, and therefore, a dual-porosity model of Durner (1994) was used to fit the water retention curve (Saedi et al. 2021). The fitted water retention curve was used to derive the air-filled porosity [AFP(h)] curve as a function of soil water potential as AFP(h) = $\theta_s - \theta(h)$, where h is the absolute matric potential, θ is the volumetric water content and θ_s is the saturated water content (Saedi et al. 2021).

Plant and treatments preparations

A pot experiment was performed with a completely randomized design and factorial arrangement of treatments. The treatments were two genotypes of tall fescue (75B and 75C) infected with two levels of endophyte infection (E+: endophyte-infected and E−: endophyte-free) and seven varying levels of air-filled porosity in the pots (AFP of 0, 0.025, 0.050, 0.075, 0.100, 0.125 and 0.150 m^3 m^{-3}). Each treatment was replicated three times (in total, 84 pots). The genotypes 75B and 75C, collected initially from Iran's natural habitats, were infected by *Epichlöe* endophytes and cloned in a field. These two genotypes were chosen based on the previous studies indicating their different morphological and physiological responses to various environmental stresses (Sabzalian and Mirlohi 2010; Zarean et al. 2017). These two genotypes are now putative clones used in many studies (e.g., Sabzalian and Mirlohi 2010; Hosseini et al. 2015a,b; Zarean et al. 2017). The E − tillers of tall fescue were chosen from vegetatively-propagated E + plants, which had been treated with a fungicide mixture containing propiconazole (1-[{2-(2, 4-dichlorophenyl)-4-propyl-1,3-dioxolan-2yl} methyl]−1H − 1, 2, 4-triazole) and tebuconazole ((RS)-1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol) at ratios of 2 g of the active ingredient and 1 mL L^{-1} of water, respectively (Sabzalian and Mirlohi 2010). Prior to planting, the presence and absence of the fungus in E + and E − clones, respectively, were verified by staining plant leaf tissues with Rose Bengal as described by Saha et al. (1988).

PVC pots (7 cm internal diameter and 12 cm height with removable bases) were filled with air-dried soil (< 4 mm) at a BD of 1.54 Mg m^{-3}. Then five tillers of each E + and E− tall fescue from both genotypes with similar length and number of leaves were selected and transplanted into each pot at a depth of 2 cm. The soil surface in each pot was covered with a 1-cm layer of gravel (2–4 mm) to minimize evaporation lost from the soil surface. During growth period (13-months), the plants were kept in a greenhouse under controlled temperature (mean day and night temperatures of 25 ± 2°C and 18 ± 2°C, respectively) and natural light intensity (a daily average of 1000 µmol m^{-2} s^{-1}).

During the first four months, plants were irrigated from the top every day to maintain the soil water content around the field capacity (θ_{FC} = water content at h of 100 hPa) by monitoring pots' weight. When plants were fully developed, the average soil water content in each pot was adjusted to a value corresponding to the selected seven varying levels of AFP (0.025, 0.050, 0.075, 0.100, 0.125, 0.150, and 0.175 m^3 m^{-3}). The soil water content $\theta(h)$ corresponding to each AFP treatment was calculated from the measured soil retention curveas AFP(h) = $\theta_s - \theta(h)$ (Saedi et al. 2021). The pots were weighed and watered from the top twice per day to adjust the soil water content. Once every two weeks, a mixture of Johnson nutrient solution of half strength (Johnson et al. 1957) and distilled water was added to the irrigation water. The diluted nutrient solution contained 8 mM NO_3^-, 3 mM K^+, 2 mM Ca^{2+}, 1 mM HPO_4^{2-} + $H_2PO_4^-$, 0.5 mM Mg^{2+} and 0.5 mM SO_4^{2-} and sufficient amount of micronutrients.

Three months later, when plants were seven months old, they were transplanted into larger pots (15 cm top diameter, 13 cm base diameter and 14 cm height) in the following way: the pots were opened from the bottom, and the entire intact root system and its surrounding soil were gently removed, and placed into larger pots and the remaining extra space was filled with similar soil to the same bulk density. A 2-cm-thick layer of gravel (2–4 mm) was added to the top of each pot to minimize evaporation. Since tall fescue had shown high tolerance to poor aeration, new AFP levels were applied as follows: 0, 0.025, 0.050, 0.075, 0.100, 0.125, and 0.150 m^3 m^{-3}. Therefore, the AFP level of 0.00 m^3 m^{-3} was added, and the AFP level of 0.175 m^3 m^{-3} was removed from the experiment.

The AFP of 0 $m^3\ m^{-3}$ was obtained by a complete saturation of the soil and maintaining a thin water layer on the surface. The AFP levels were monitored daily (gravimetrically) and maintained for a period of another six months.

Characterization of soil aeration status of different treatments

The aeration status of different tested AFPs was evaluated by measuring air permeability (k_a), gas diffusion coefficient (D_p), redox potential (Eh), and pH of soil. k_a and D_p were measured using the standard methods on soil samples prepared in the same condition as the pots were filled with soil, and their water contents were adjusted. The details of air permeability and gas diffusion coefficient measurements are given in Saedi et al. (2021).

Oxidation-reduction conditions are commonly assessed by measuring the redox potential (Eh, expressed in volts). Here we measured the pH and the Eh of the root zone at the end of the experiment, before the plant harvest, using a voltmeter (model CP511, Elmetron Company, Zabrze, Poland) and a combined platinum reference electrode (Patrick et al. 1996). The Eh and pH electrodes were inserted into the soil at a depth 5 cm, and their readings were averaged at three different locations of each pot as the representative of Eh and pH of the root zone. To relate the Eh to electron activity in the soil, some researchers suggested expressing the values of Eh as pe (electrical potential, defined as $pe = -\log_{10}[e^-] = Eh(mV)/59.2$ where e^- is the activity of electrons). In this representation, pe becomes a non-dimensional property like pH (Truesdell 1968). As the numerical value of Eh depends on the pH, Lindsay and Sadiq (1983) proposed using pe + pH as an indicator for soil redox potential.

Plant growth and its morphological, anatomical, and biochemical properties

Leaf relative water content, water potential, and chlorophyll fluorescence

A few days before the plant harvest (six months after applying different AFP treatments in large pots, when plants were 13 months old and were exposed to different AFP treatments for 9 months in total), the leaf relative water content (RWC), leaf water potential (LWP) and leaf chlorophyll fluorescence (Chlo *fl*) were determined. To do so, a few leaves were cut, and we determined the RWC gravimetrically and the LWP using a pressure bomb apparatus (Model 3115, Soil Moisture Equipment Corp., Santa Barbara, California, USA), following standard procedures (Kirkham 2014). The chlorophyll fluorescence (Chlo *fl*) was measured with a Plant Efficiency Analyzer (Hansatech instruments, England) following the procedure of Maxwell and Johnson (2000).

Shoot and root biomass

Plant shoots and roots in each pot were harvested six months after applying different AFP treatments in large pots. The fresh shoot weight was recorded first and then divided into three groups: some parts were transferred to the laboratory for determination of carotenoids and chlorophyll contents; the second parts were frozen in liquid nitrogen and stored at −40°C for measuring the antioxidant enzyme activities, and the rest were dried in an oven at 70°C for determination of the shoot dry weight. The roots were gently removed from the soil by opening the removable base of the pots and were washed with tap water over a sieve with a mesh size of 0.5-mm. Then the fresh root weight was determined gravimetrically and the root volume was calculated by the water displacement method (Harrington et al. 1994). Subsamples of the fresh roots were frozen in liquid nitrogen and stored at − 80°C for measuring the ADH activity as will be further explained later. Some young roots were also sampled and placed in fixation solution (glycerol, alcohol, and distilled water in equal volumetric ratios, 1:1:1) to study the formation of aerenchyma across the root tissue; the details of the method are given in the subsection “Anatomical observations of roots”.

Carotenoids and chlorophyll contents

The concentrations of carotenoids (Carot), total chlorophyll (Chlo *T*), chlorophyll *a* (Chlo *a*) and chlorophyll *b* (Chlo *b*) of fully-expanded leaves were determined as described by Arnon (1967): 0.2 gram of leaf samples were homogenized in a mortar, extracted with 10 mL of 80% acetone solution and then filtered through Whatman No. 42 filter paper. The absorbance of the extract was recorded at 470 nm (A_{470}), 663 nm (A_{663}), and 645 nm (A_{645}) wavelengths using a Jenway 6505 UV/Vis spectrophotometer (Bibby Scientific Limited, Staffordshire, UK) and the carotenoids and chlorophyll contents were calculated using the following equations:

$$\text{Chlo } a\ (\text{mg/g tissue}) = \frac{[12.7(A_{663}) - 2.69(A_{645})] \times V}{1000 \times W} \quad (1)$$

$$\text{Chlo } b\ (\text{mg/g tissue}) = \frac{[22.9(A_{645}) - 4.68(A_{663})] \times V}{1000 \times W} \quad (2)$$

$$\text{Chlo } T\ (\text{mg/g tissue}) = \frac{[20.2(A_{645}) + 8.02(A_{663})] \times V}{1000 \times W} \quad (3)$$

$$\text{Carot (mg/g tissue)} = \frac{100(A_{470}) - 3.27(\text{mg Chlo } a) - 104(\text{mg Chlo } b)}{227} \quad (4)$$

where V is the final volume (mL) of chlorophyll extract in 80% acetone, and W is the fresh weight (g) of the extracted tissue (Arnon 1949).

Antioxidant enzymes activities in leaves

The crude extract for CAT and APX measurements was isolated as follows: 0.1 gram of fresh leaves were homogenized with 1 mL of phosphate buffer solution (PBS, 50 mM, pH 7.0) containing NaH_2PO_4, Na_2HPO_4, 2 mM ethylenediaminetetraacetic acid (EDTA) and 0.1% X-Triton 100(v/v). The homogenate was centrifuged at 12000 × *g* for 20 min at 4°C and then the supernatant was used for enzyme activity assays (Dhindsa 1981).

Catalase (EC 1.11.1.6) activity was determined following the consumption of H_2O_2 (extinction coefficient 39.4 mM cm^{-1}) at 240 nm for 30 s according to the protocol of Aebi (1984). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 15 mM H_2O_2 and 50 µL enzyme extract in a 3 mL volume.

Ascorbate peroxidase (EC 1.11.1.11) activity was determined as described by Nakano and Asada (1981). The reaction solution contained 50 mM PBS, 0.1 mM H_2O_2, 0.5 mM ascorbate (extinction coefficient 2.8 mM cm^{-1}), 0.1 mM EDTA and 150 µL of the enzyme extract. The absorbance of the supernatant was read at 290 nm, 70 s after the addition of H_2O_2 to the solution.

Activity of alcohol dehydrogenase activity in roots

To examine variation in ADH activity among different tested genotypes, crude extracts were obtained from the root of plants exposed to varying AFP. Root tissue (0.5 g) was first powdered with liquid nitrogen and then homogenized with 5.0 mL of extraction buffer consisted of 50 mM Tris-HCl and 10 mM DTT, pH 8.0. The extract was centrifuged at 12000 *g* for 15 min at 4°C in a refrigerated centrifuge (Chung and Ferl 1999) and the supernatant was collected and used immediately for enzyme activity assays.

The spectrophotometric measurements of the reduction of NAD to NADH in the presence of ethanol substrate were used to quantify differences in ADH activity among genotypes in flooding and non-flooding environments by applying a modified version of methods described by Chung and Ferl (1999) and Liu et al. (2012). A 50 µL of enzyme extract and 900 µL of reaction solution composed of 50 mM Tris-HCl (pH 8.0) and 1 mM NAD were incubated in microcentrifuge tubes in a water bath at 30°C for 3 min. Then, 50 µL 95% ethanol and the previously described 950 µL enzyme and reaction solution were added directly to a cuvette. The increase in absorbance at 340 nm was recorded every 30 s for 3 min with a Jenway 6505 UV/Vis spectrophotometer (Bibby Scientific Limited, Staffordshire, UK) to determine the concentration of NADH. A unit of ADH activity was defined as the production of 1 µmol NADH min^{-1} g^{-1} fresh weight of root (abbreviated as U g FW^{-1}).

Anatomical observations of roots

Root segments were prepared from the apical (within 30 mm of the root tip) regions of tall fescue roots. Cross-sections were prepared by hand-sectioning with a razor blade. Each section was photographed using a polarized light microscope (BH 12, OLYMPUS) equipped with a digital camera. The percentage of root cross-section occupied by aerenchyma was determined using a program written in Matlab 2014 software (Mathworks Corporation, US). Anatomical observations of root cross-sections were conducted using four combinations of plant genotype and endophyte status (E + 75B, E− 75B, E + 75C, and E− 75C) at two levels of AFP out of seven (i.e., 0.00 and 0.150 m^3 m^{-3}).

Statistical analysis

A completely randomized design with a factorial arrangement was used to examine the effect of different tested treatments and their combinations (2 genotypes × 2 levels of endophyte status × 7 levels of AFP) on some plant growth parameters, plant physiological parameters, the activity of antioxidant enzymes and fermentation enzyme (ADH) all in three replicates. Analysis of variance (ANOVA) was conducted using the SAS software (SAS Institute, v. 9.1, Cary, North Carolina, US). To evaluate the redox parameters (i.e., pH, Eh, and pe + pH) of the root zone compared to bulk soil, an ANOVA was conducted using four combinations of plant genotypes and endophyte status (E + 75B, E− 75B, E + 75C, E− 75C) and bulk soil (in total, five levels), at 3 levels of AFP out of 7 (i.e., 0.00, 0.025 and 0.050 m^3 m^{-3}) according to a completely randomized design with a factorial arrangement. In addition, another ANOVA was conducted to examine the effect of the same four combinations at 2 levels of AFP out of 7 (i.e., 0.00 and 0.150 m^3 m^{-3}) on the percentage of aerenchyma formation in the roots according to a completely randomized design with a factorial arrangement. Mean comparisons among different treatments were performed using the LSD test at $p < 0.05$. Pearson pair-wise correlations were used to determine and interpret the relations between plant growth, physiological parameters, and enzyme activities.

Results

Soil aeration status of different treatments

The values of intrinsic air permeability (k_a) and soil-gas diffusion coefficient (D_p) at different AFP levels are presented in Table 1 of Saedi et al. (2021). It is interesting to note that critical k_a (i.e., 2 μm^2) occurred at AFP of 0.05 m^3 m^{-3} with D_P/D_0 of 0.0029 in the studied soil. In this study, the AFP ranges of 0.150–0.10, 0.075–0.05, and 0.025–0.00 m^3 m^{-3} were considered, respectively, as non-, medium-, and high-limiting oxygen ranges.

Table 1

Analysis of variance (ANOVA) of air-filled porosity (AFP), tall fescue genotype (G), and endophyte status (E) effects on the dry weight of root (DW_{root}), dry weig the ratio of dry weights of root to shoot (Root/Shoot), root volume (RV), dry weight of whole plant (DW_{plant}), relative water content (RWC), absolute leaf wate chlorophyll *a* (Chlo *a*), chlorophyll *b* (Chlo *b*), total chlorophyll (Chlo *T*), chlorophyll fluorescence (Chlo *fl*), and catalase enzyme (CAT), ascorbate peroxidase alcohol dehydrogenase enzyme (ADH) activities.

Source of variance	Mean Square												
	df	DW_{root}	DW_{shoot}	Root/Shoot	RV	DW_{plant}	RWC	|LWP|	Chlo *a*	Chlo *b*	Chlo *T*	Chlo *fl*	CAT
AFP	6	37.19^{**}	1.44^{ns}	2.96^{**}	424.03^{**}	27.97^{*}	13.47^{**}	2.73^{*}	0.1890^{**}	0.068^{**}	0.469^{**}	0.0011^{**}	0.156^{**}
G	1	37.45^{**}	2.95^{ns}	0.03^{ns}	510.11^{*}	61.42^{*}	17.13^{**}	0.69^{ns}	0.0089^{ns}	0.029^{**}	0.065^{*}	0.0028^{**}	0.001^{ns}
E	1	0.40^{ns}	64.84^{**}	42.02^{**}	613.44^{*}	55.03^{*}	5.84^{*}	0.08^{ns}	0.4954^{**}	0.034^{**}	0.667^{**}	0.0002^{ns}	0.175^{**}
AFP × G	6	0.28^{ns}	0.46^{ns}	0.41^{ns}	37.38^{ns}	0.34^{ns}	0.67^{ns}	1.72^{ns}	0.0009^{ns}	0.002^{ns}	0.003^{ns}	0.0002^{ns}	0.002^{*}
AFP × E	6	4.29^{ns}	1.60^{ns}	1.11^{ns}	36.83^{ns}	3.03^{ns}	0.46^{ns}	0.76^{ns}	0.0109^{ns}	0.002^{ns}	0.019^{ns}	0.0002^{ns}	0.013^{**}
G × E	1	602.62^{**}	6.52^{*}	4.74^{*}	23971^{**}	734.50^{**}	1.50^{ns}	4.49^{*}	0.0001^{ns}	0.007^{ns}	0.001^{ns}	0.0011^{ns}	0.019^{**}
AFP × G × E	6	2.03^{ns}	0.87^{ns}	0.90^{ns}	144.19^{ns}	2.46^{ns}	1.58^{ns}	0.45^{ns}	0.0050^{ns}	0.003^{ns}	0.019^{ns}	0.0028^{ns}	0.004^{**}
Error	-	2.28	1.25	0.86	9.41	3.12	1.14	3.01	0.095	0.052	0.122	0.016	0.024
CV%	-	10.52	24.35	19.20	14	11.65	1.22	14.57	13.83	14.26	11.58	2.05	14.99
R^2	-	0.76	0.53	0.65	0.85	0.66	0.63	0.41	0.77	0.78	0.82	0.48	0.97

ns, * and ** stand for non-significant, significant at 0.05 and 0.01 probability levels, respectively; CV is coefficient of variation.

The ANOVA showed that the redox parameters (Eh, pH and pe + pH) were significantly affected by the plant genotypes, endophyte status, AFP level, and interactions. Mean comparisons of Eh, pH and pe + pH in the bulk and root zone soils at three selected AFP levels (i.e., interaction of the treatments) are shown in Fig. 1. The values of Eh were significantly greater in the root zone soil of all treatments compared to one of their bulk soils. The value of Eh in the root zone of all treatment was positive, while it was negative in their bulk soil (expect AFP of 0.05 $m^3\ m^{-3}$). Negative Eh values represent high electron activity, and intense anaerobic conditions and its positive values stand for low electron activity and aerobic conditions or moderately anaerobic conditions (Husson, 2013). The Eh did not show any significant difference between the root zone soils of 75B and 75C genotypes, either endophyte-infected (E+) or endophyte-free (E–) at different AFP levels except for the root zone of E + 75C under saturated condition (Fig. 1a).

The pH of both root zone and bulk soil did not show any remarkable difference between different treatments and remained near neutrality (Fig. 1b). Indeed, we did not expect a noteworthy difference among treatments due to the buffering capacity of the studied soil originated from its carbonates. The trend of pe + pH was similar to that of Eh (Fig. 1c).

Adventitious roots

During plant growth, we observed a remarkable formation of adventitious roots (shallow roots growing) at the soil surface of plant genotype without endophyte infection (E– 75C, E– 75B), while it was less pronounced for the case of plant genotype infected with endophyte (E + 75C and E + 75B) (Fig. 2). In the E– 75B plants, the formation of adventitious roots began from AFP of 0.050 $m^3\ m^{-3}$ (in the medium-limiting oxygen range) and covered the entire soil surface under anoxic conditions (i.e., AFP = 0). However, very few adventitious roots were observed in the E + 75B plants only under fully saturated conditions. In E– 75C, adventitious roots were seen in the AFP levels of 0.025 and 0.00 $m^3\ m^{-3}$ (i.e., high-limiting oxygen range).

Plant growth parameters

Analysis of variance (ANOVA) of the effects of treatments on different plant parameters is presented in Table 1. The main and interaction effects of treatments on fresh shoot weight were not significant; therefore, its results were not presented. Although the dry weight of shoot (DW_{shoot}) was not significantly affected by AFP, the AFP had a significant effect on the dry weight of root (DW_{root}), ratio of dry weights of root to shoot (root/shoot), root volume (RV) and the dry weight of plant (DW_{plant}).

The DW_{root} and root/shoot ratio significantly decreased with decreasing AFP levels from 0.075 $m^3\ m^{-3}$ to zero (in the limiting oxygen range), but no significant differences were observed between the mean values of DW_{root} and root/shoot as the AFP decreased in the non-limiting oxygen range from 0.150 to 0.075 $m^3\ m^{-3}$ (Table 2). The mean values of RV and DW_{plant} significantly decreased at AFP of 0.025 and 0.00 $m^3\ m^{-3}$ (i.e., high-limiting oxygen range), but the changes were insignificant with a decrease of AFP from 0.150 to 0.025 $m^3\ m^{-3}$ (Table 2). Under full saturated condition (anoxia) during a 9-months period, relative decreases of 19.4, 20.1, 21.8, and 14.0 % were observed for DW_{root}, RV, root/shoot ratio, and DW_{plant}, respectively, when compared to good aeration condition with AFP of 0.150 $m^3\ m^{-3}$ (Table 2). The mean values of plant growth parameters (i.e., DW_{root}, RV, root/shoot ratio and DW_{plant}) in the non-limiting oxygen range were 23.33 g per pot, 70.73 cm^3 per pot, 70.69 and 28.25 g per pot, respectively. In contrast, the corresponding values in the high-limiting oxygen range were 19.5 g per pot, 57.67 cm^3, 59.16 and 24.93 g per pot.

Table 2
Mean comparisons of the effects of soil air-filled porosity (AFP), endophyte status (E + and E−) and tall fescue genotype (75B and 75C) on the dry weight of root (DW_{root}), root volume (RV), the ratio of dry weights of root to shoot (Root/Shoot), dry weight of plant (DW_{plant}).

Treatments		DW_{root}	RV	Root/Shoot	DW_{plant}
		(g per pot)	(cm^3 per pot)	(−)	(g per pot)
AFP ($m^3\ m^{-3}$)	0.000	19.06^{c}	56.25^{c}	56.25^{c}	24.48^{c}
	0.025	19.93^{bc}	59.08^{b}	59.08^{b}	25.37^{bc}
	0.050	21.06^{b}	64.58^{ab}	64.58^{ab}	26.68^{abc}
	0.075	21.68^{ab}	64.75^{ab}	64.75^{ab}	26.43^{abc}
	0.100	23.12^{a}	69.33^{a}	69.33^{a}	28.27^{a}
	0.125	23.22^{a}	70.83^{a}	70.83^{a}	28.00^{ab}
	0.150	23.64^{a}	71.92^{a}	71.92^{a}	28.48^{a}
Endophyte	E+	21.74^{a}	62.55^{b}	62.55^{b}	26.00^{b}
	E−	21.60^{a}	67.95^{a}	67.95^{a}	27.62^{a}
Genotype	75B	22.34^{a}	67.71^{a}	67.71^{a}	27.67^{a}
	75C	21.00^{b}	62.79^{a}	62.79^{a}	25.96^{b}

In each column and each group, means with different letters are significantly different (LSD, $p < 0.05$).

Plant growth parameters (i.e., DW_{root}, root/shoot, RV, DW_{plant} and FW_{plant}) were significantly affected by the presence of endophyte (Table 1). A significant interaction of endophyte × genotype indicated that the effect of endophyte presence on plant growth response (i.e., DW_{root}, RV and DW_{plant}) was dependent on the plant genotype (Fig. 3). In the 75B genotype, the mean values of DW_{root}, RV and DW_{plant} were significantly greater in the E + plants than those of E− plants. However, in the 75C genotype, they were lower in E + plants compared to E− ones (Fig. 3a,b,d). The mean of root/shoot ratio was significantly greater in the E + plants than in the E− plants in both genotypes, although the difference between E + and E− plants was greater in the 75C genotype (Fig. 3c).

Plant physiological parameters

The ANOVA showed that the effect of AFP on the RWC and |LWP| was significant. The changes of AFP from 0.150 to 0.050 $m^3\ m^{-3}$ (i.e., non and medium-limiting oxygen ranges) had no significant effect on the RWC; however, RWC significantly decreased with a further reduction of AFP from 0.050 to 0.0 $m^3\ m^{-3}$ (i.e., high-limiting oxygen range) (Table 3). The mean value of RWC was greater in the E + tall fescue than the E− plant, and it was higher in the 75B genotype than the 75C genotype. No significant difference was observed in |LWP| values as the AFP changed from 0.125 to 0.0 $m^3\ m^{-3}$, but there was a significant difference in mean values of |LWP| between AFP levels of 0.150 (control) and 0.125 $m^3\ m^{-3}$ (Table 3). The genotype and presence of endophyte significantly affected RWC but had no significant effect on the |LWP| (Tables 1 and 3).

Table 3
Means' comparisons of the effects of soil air-filled porosity (AFP), endophyte status (E + and E−) and tall fescue genotype (75B and 75C) on the relative water content (RWC), absolute leaf water potential (|LWP|), chlorophyll *a* (Chlo *a*), chlorophyll *b* (Chlo *b*), total chlorophyll (Chlo *T*), chlorophyll fluorescence (Chlo *fl*) and catalase enzyme (CAT), ascorbate peroxidase enzyme (APX) and alcohol dehydrogenase enzyme (ADH) activities.

Treatments		RWC	|LWP|	Chlo *a*	Chlo *b*	Chlo *T*	Chlo *fl*	CAT	APX	ADH
		(%)	(MPa)	(mg g FW^{-1})	(mg g FW^{-1})	(mg g FW^{-1})	(−)	(U g FW^{-1})	(U g FW^{-1})	(U g FW^{-1})
AFP	0.000	91.32^{c}	2.25^{a}	0.49^{c}	0.25^{f}	0.74^{d}	0.75^{c}	0.36^{a}	5.08^{a}	2.60^{a}
(m^3 m^{-3})	0.025	91.41^{c}	2.11^{a}	0.55^{c}	0.30^{e}	0.85^{c}	0.76^{bc}	0.26^{b}	3.91^{b}	1.52^{b}
	0.050	92.36^{b}	2.09^{a}	0.66^{b}	0.34^{de}	0.99^{b}	0.77^{ab}	0.19^{c}	3.15^{c}	1.19^{c}
	0.075	93.26^{ab}	2.10^{a}	0.75^{a}	0.37^{dc}	1.13^{a}	0.77^{ab}	0.12^{d}	1.47^{d}	0.65^{d}
	0.100	93.70^{a}	2.13^{a}	0.80^{a}	0.41^{bc}	1.21^{a}	0.77^{ab}	0.07^{e}	0.76^{e}	0.37^{e}
	0.125	93.41^{a}	1.97^{ab}	0.80^{a}	0.42^{ba}	1.23^{a}	0.77^{ab}	0.06^{e}	0.73^{e}	0.20^{e}
	0.150	93.87^{a}	1.80^{b}	0.76^{a}	0.47^{a}	1.23^{a}	0.78^{a}	0.06^{e}	0.63^{e}	0.21^{e}
Endophyte	E+	93.03^{a}	2.04^{a}	0.76^{a}	0.38^{a}	1.1^{a}	0.77^{a}	0.11^{b}	1.9^{b}	0.97^{a}
	E−	92.46^{b}	2.09^{a}	0.61^{b}	0.34^{b}	0.96^{b}	0.76^{a}	0.21^{a}	2.6^{a}	0.96^{a}
Genotype	75B	93.22^{a}	2.02^{a}	0.70^{a}	0.38^{a}	1.08^{a}	0.77^{a}	0.16^{a}	1.96^{b}	0.96^{a}
	75C	92.26^{b}	2.11^{a}	0.679^{a}	0.346^{b}	1.02^{b}	0.758^{b}	0.157^{a}	2.53^{a}	0.975^{a}

In each column and each group, means with different letters are significantly different (LSD, $p < 0.05$).

The main and interaction effects of treatments on Carot content were not significant; therefore, its results were not presented. Instead, soil aeration significantly affected the concentrations of all chlorophyll types (Table 1). No significant difference was observed for the Chlo *T* and Chlo *a* by changing the AFP from 0.150 to 0.075 m^3 m^{-3} (i.e., non-limiting oxygen range), but with further reduction in AFP to 0.050 m^3 m^{-3}, their decreases became significant. Concentrations of Chlo *T* and Chlo *a* significantly decreased as the AFP decreased from 0.050 to 0.0 m^3 m^{-3} (i.e., high-limiting oxygen range). No significant difference was observed in the concentrations of Chlo *b* between the AFP values of 0.150 and 0.125 m^3 m^{-3}, but with a further reduction of AFP from 0.100 to 0.0 m^3 m^{-3} (in the limiting oxygen range), it gradually and significantly decreased (Tables 1 and 3).

A significant difference was observed in Chlo *fl* concentration only between two AFP levels of 0.025 and 0.0 m^3 m^{-3} (i.e., high-limiting oxygen range) compared to control (i.e., AFP = 0.015 m^3 m^{-3}). There was no significant difference in Chlo *fl* between other AFP levels compared with control (Table 3). Generally in the lack of oxygen (full saturation), the mean values of Chlo *a*, Chlo *b*, Chlo *T* and Chlo *fl* decreased by 36.0, 46.0, 39.8 and 3.7 %, respectively, compared to good aeration condition (i.e., AFP = 0.150 m^3 m^{-3}) (Table 3). The mean values of Chlo *T* in the non-, medium-, and high-limiting oxygen ranges were 1.22, 1.06 and 0.79 mg g FW^{-1}, respectively. The corresponding values for Chlo *fl* were 0.77, 0.77 and 0.75.

Endophyte and genotype significantly affected the concentrations of chlorophyll types (Table 1). The Chlo *a*, Chlo *b*, Chlo *T* and Chlo *fl* were significantly greater in the E + plants than those in the E− plants and were more in the 75B genotype compared to 75C genotype (Table 3).

Enzymes activities

The triple interaction effects of endophyte status, genotype and AFP on the enzyme activities (CAT, APX and ADH) were significant (Table 1). The soil air-filled porosity had a significant effect on the activities of antioxidative enzymes (CAT and APX) in the leaves of all combinations of plant genotypes and endophyte status conditions (Fig. 4a,b). The maximum activities of CAT and APX were observed under full saturated condition (AFP = 0.00) in both genotypes infected with endophyte (E+) or endophyte-free (E−). With decreasing the AFP to values lower than 0.075 m^3 m^{-3}, the CAT activity increased in the E− plants of both genotypes. Whereas APX activity significantly increased in the E− 75B plants only at the saturated condition. However, when AFP decreased to the values below 0.050 m^3 m^{-3}, the CAT activity in E + plants of both genotypes and the APX activity in E + 75B plants significantly increased (Fig. 4a,b). Different thresholds were observed for the 75C genotype; significant increases in the APX activity for E− and E + plants were observed in the AFP levels lower than 0.025 m^3 m^{-3} and 0.05 m^3 m^{-3}, respectively (Fig. 4b).

With decreasing AFP from 0.150 to 0.100 m^3 m^{-3} (in the non-limiting oxygen range), no significant differences in the CAT and APX activities were observed among the E− and E + plants of both genotypes. However, for the AFP values below 0.100 m^3 m^{-3} (in the limiting oxygen range), the CAT and APX activities were significantly greater in the E− plants compared to those in the E + plants (Fig. 4a,b). Among the E− plants, the CAT activity was greater in the 75B genotype compared to the 75C genotype, whereas the APX activity was significantly higher in the 75C genotype compared to the 75B genotype under oxygen-limited conditions (especially at the AFP levels of 0.025 and 0.00 m^3 m^{-3} (Fig. 4a,b). In contrast, the lowest activities of antioxidant enzymes (CAT and APX) were observed in the E + 75B plants at the AFP values lower than 0.025 m^3 m^{-3} (high-limiting oxygen range).

The mean values of CAT activity in high-, medium- and non-limiting oxygen ranges for E + 75B and E− 75B plants were 0.193, 0.088 and 0.054 U g^{-1} FW, and 0.420, 0.261 and 0.071 U g^{-1} FW, respectively. The corresponding values for the E + 75C and E− 75C plants were 0.271, 0.090 and 0.055 U g^{-1} FW, and 0.349,

0.179 and 0.086 U g^{-1} FW, respectively (Fig. 4a). The mean values of APX activity in the high-, medium- and non-limiting oxygen ranges for E + 75B and E– 75B plants were 3.46, 1.23 and 0.54 U g^{-1} FW, and 4.65, 2.67 and 0.62 U g^{-1} FW, respectivity. The corresponding values for the E + 75C and E– 75C plants were 4.17, 2.96 and 0.50 U g^{-1} FW, and 5.69, 2.38 and 1.17 U g^{-1} FW, respectivity (Fig. 4b).

Air-filled porosity (AFP) significantly affected the activity of ADH enzyme in the roots of both genotypes infected with endophyte or endophyte-free (Fig. 4c). The maximum values of ADH activity were observed under fully saturated conditions (Fig. 4c). In E + plants of both genotypes, as AFP reached below 0.075 m^3 m^{-3} (i.e., the limiting oxygen range), a significant increase in the ADH activity was observed toward saturated condition. However, a significant increase in the ADH activity of E– plants for both genotypes of 75B and 75C was observed when AFP was less than 0.025 m^3 m^{-3} (i.e., high-limiting oxygen range) (Fig. 4c). An upward trend in the mean values of ADH activity with decreasing AFP, indicates a shift from aerobic metabolism to anaerobic (fermentation pathway) in tall fescue.

Significant differences were observed in the ADH activity values among the E– and E + plants of both genotypes with decreasing AFP from 0.025 to 0.00 m^3 m^{-3} (in the high-limiting oxygen range). Besides, the ADH activity was significantly greater in the E + plants than that in the E– plants for the 75C genotype and was lower in the E + plants compared to the E– ones for the 75B genotype under full saturated condition (Fig. 4c).

The mean values of ADH activity in the high-, medium- and non-limiting oxygen ranges for E + 75B and E– 75B plants were 1.80, 0.80 and 0.26 U g^{-1} FW, and 2.29, 1.06 and 0.24 U g^{-1} FW, respectively. The corresponding values for the E + 75C and E– 75C plants were 2.30, 0.96 and 0.32 U g^{-1} FW, and 1.84, 0.86 and 0.20 U g^{-1} FW, respectively.

Aerenchyma

Waterlogging (full saturated condition, AFP = 0.00) during a 9-month period altered the anatomy of tall fescue roots (Fig. 5). The aerenchyma formation was significantly increased in the roots of both genotypes (75B and 75C) infected with endophyte (E+) or endophyte-free (E–) under oxygen-limited conditions, but the percentages of aerenchyma were significantly greater in the E– plants compared to E + plants (Fig. 5i), especially in the genotype 75C.

Discussion

Plant growth parameters

Our results showed that plant roots had a remarkable effect on the soil redox parameters (Eh and pe + pH) of their surrounding soil (the root zone) under oxygen-limited conditions (Fig. 1a,c). This alteration can be a direct effect of root exudation or an indirect effect through the development of specific microorganisms altering the Eh and pH (Hartmann et al. 2009). The roots of some plants under anaerobic conditions can obtain essential oxygen through air-filled intercellular spaces in the roots and shift the Eh values of root zone toward optimum values (Husson 2013). For instance, in anaerobic conditions, rice roots were able to raise the Eh from + 120 mV in the bulk soil to + 420 mV at the root surface (Flessa and Fischer 1992). Figure 1 confirmed that the tall fescue was able to shift the Eh values of the root zone toward optimum values by aerenchyma formation, as shown in Fig. 5.

To date, few studies have evaluated the effects of grass-*Epichlöe* symbiota on plant response to oxygen-limited conditions (Adams et al. 2017; Arachevaleta et al. 1989; Song et al. 2015). Overall, the results of these studies showed that oxygen deficiency negatively affected plant performance. For example, Song et al. (2015) suggested that waterlogging reduces the shoot and root biomasses, the number of tillers, shoot height, and root length of *H. brevisubulatum* plants. In line with the results of Arachevaleta et al. (1989), we did not observe any remarkable difference between growth parameters of E + and E– tall fescue plants (both genotypes) with decreasing AFP from 0.150 to 0.050 m^3 (Table 2). Tall fescue maintained nearly 80–86% of its growth under completely waterlogged conditions during a 9-month period (Table 2). In addition, there was no significant alteration in shoot growth under anoxic conditions (Table 1). Thus, regardless of the endophyte status, both tested plant genotypes were tolerant to poor aeration conditions.

In contrast to the findings of Arachevaleta et al. (1989) that address the ineffectiveness of endophyte symbiosis on plant growth under anoxic condition, the results of Song et al. (2015) indicated that in flooded *H. brevisubulatum*, endophyte presence was associated with greater root vitality, root biomass and tiller production, along with lower leaf wilt rates, than that occurred in E– plants. In this study, we found that the positive impact of endophyte symbiosis under oxygen-deficiency (hypoxia) and lack of oxygen (anoxia) conditions is dependent on the tall fescue genotype. In the 75B genotype, the mean values of DW_{root} and DW_{plant} were greater in the E + plants than those in the E– plants. However, in the 75C genotype, growth parameters were lower in the E + plants than the E– ones (Fig. 3). Such a discrepancy in the reaction of the two genotypes has also been reported in the previous studies. For instance, Zarean et al. (2017) showed that the flag leaf greenness in the 75C genotype was lower in the E + tall fescues compared to the E– ones, whereas in the 75B genotype, it was higher in the E + plant. In addition, their results suggested that endophyte infection almost increased seed production in both genotypes of 75C and 75 B, but positive effects of endophyte were greater in 75B genotype.

The root/shoot ratio was significantly greater in the E + plants than in E– plants in both plant genotypes (Fig. 3c), whereas for the other growth parameters, the significant interaction of endophyte × genotype indicated that the effect of endophyte presence on plant growth was dependent on the plant genotype (Table 1, Fig. 3). But changes in the root/shoot ratio in both E + and E– plants were not related to the AFP (Table 1). These results were contrary to the results of Adams et al. (2017). They observed that under flooding, endophyte (*Epichloë* sp.) presence increased the root-to-shoot ratio of *P. leptocoma* by 58% relative to E– plants, whereas under non-flooding conditions, the root-to-shoot ratio was only 9% higher in the E + plants. Hesse et al. (2005) found that the root dry weight of two genotypes of *Lolium perenne* L. significantly increased by 49% due to the presence of endophyte, particularly in the flooding treatment. It is likely that endophyte inhibited the decline in plant shoot growth and increased plant resistance to oxygen deficiency through the expansion of the root system. Therefore, the endophyte symbiosis through alteration of the root system of the host plant provides a benefit to plant growth and improves the uptake of water

and nutrients, especially phosphorus, under the limited-oxygen conditions. Previous studies (Malinowski and Belski 2000) also showed that the presence of endophytes could increase the uptake of water and soil nutrients, including calcium, phosphorus under environmental stresses.

Adventitious roots (morphological adaptation)

The emergence of adventitious roots in many species, e.g., rice (Justin and Armstrong 1991), barley (Zhang et al. 2015) and tomato (Else et al. 2008), were observed as a morphological adaptation under oxygen-limited conditions. Therefore, the high ability to produce adventitious roots in the studied genotypes of tall fescue (especially in the E– plants, see Fig. 2) is commonly related to enhanced tolerance to anoxia and hypoxia. More evidence of adventitious root formation near the soil surface of endophyte-free plants may suggest the importance of this root feature as an adaptive strategy of these plants to overcome severe oxygen-limited conditions. In contrast, in the case of endophyte-infected plants, they may benefit more efficiently from a companion of endophyte and therefore do not need to invest their photosynthetic products on the development of such roots.

Plant physiological parameters

The |LWP| significantly increased in AFP of 0.100 $m^3\ m^{-3}$ and was lower compared to AFP of 0.150 $m^3\ m^{-3}$ (Table 3). Although when AFP reached below 0.05 $m^3\ m^{-3}$, there was a significant decrease in the RWC, the mean values of RWC in all of the aeration treatments were always above 90% (Table 3). Therefore, the water status of tall fescue was not greatly affected by the soil oxygen content. With the presence of endophyte and change of tall fescue genotype under anaerobic conditions, a slight change in the RWC was observed (Table 3). Thus, little but significant change in |LWP| and absence of significant changes in RWC over a nine-month period of flooding indicates that generally, the tall fescue plant is resistant to oxygen deficiency. It seems that unlike flooding-sensitive species, root hydraulic conductivity and water uptake of tall fescue were not significantly reduced under oxygen-limited conditions.

Despite the apparent water availability under flooded conditions, a reduction in leaf RWC was reported in the previous studies, e.g., for sesame (Anee et al. 2019), mung beans (Kumar et al. 2013) and tomato (Yiu et al. 2011). Under flooding stress, flooding-sensitive species become wilted due to the decrease in root hydraulic conductivity and consequently reduction of root water uptake (Kramer and Jackson 1954).

In our study, Chlo *T* (*a* + *b*) concentration began to decrease in tall fescue plants when AFP was less than 0.075 $m^3\ m^{-3}$ (Table 3). Thus, lack of oxygen can be a limiting factor for tall fescue plants' photosynthetic activity when the AFP drops below 0.075 $m^3\ m^{-3}$. Sairam et al. (2009) stated that 4 and 8 days of waterlogging in the mung bean lowered chlorophyll concentrations. Interstingly, the chlorophyll concentrations increased again following soil drainage processes, indicating a partial restoration of the photosynthetic machinery as oxygen was re-entered to the wet soil. Moreover, Chlo *a* decrease was greater than Chlo *b*, in agreement with previous studies on *Hibiscus esculentus* (Ashraf and Arfan 2005) and on *Salix integra Thunb* (Zhao et al. 2014). The Chlo *T*, Chlo *a* and Chlo *b* and Chlo *fl* were significantly greater in the E + plants compared to E– ones (Table 3) in agreement with the findings of Song et al. (2015), who found that flooding decreased the chlorophyll content of both E + and E– *H. brevisubulatum* plants, but the chlorophyll content of E + plants was greater than that of E– plants. Our results suggested that the flooding influenced the tall fescue's photosynthetic performance, but E + plants had better photosynthetic performance than E– ones (Table 3). The endophyte infection can promote plant resistance to oxygen-limited conditions. Among the studied genotypes, photosynthetic performance (Chlo *T*, Chlo *a* and Chlo *fl* contents) of the genotype 75B was better than the genotype 75C, indicating its greater resistance to oxygen deficiency (Table 3).

Enzymes activity

Any disturbance in the photosynthetic activity can increase the formation of reactive oxygen species (ROS) and, as a consequence, severe damage to membranes, DNA, proteins, and oxidative stress induction (Foyer et al. 1994). To protect the cells against ROS, plant tissues use the scavenging system of ROS that is comprised of several enzymes often with synergetic action (e.g., SOD, CAT, APX, and GR) (Yiu et al. 2011; Yordanova et al. 2004).

In our study, CAT and APX activities in the leaves of both 75B and 75C genotypes (regardless of endophyte status) significantly increased when the soil AFP reached approximately below the level of 0.075 $m^3\ m^{-3}$ (Fig. 4a,b), indicating the occurrence of oxidative stress. Our results are in line with others (Yiu et al. 2011; Yordanova et al. 2004; Zhang et al. 2015).

The E– plants of both genotypes had higher CAT and APX activities than E + ones under oxygen-limited conditions. In the high-limiting oxygen range, the highest CAT activity was observed in the E– 75B plants, whereas the maximum APX activity was observed in the E– 75C plants. In contrast, the lowest activities of antioxidant enzymes (CAT and APX) were observed in the E + 75B plants in the same condition. However, the change in trends of CAT and APX activities in the E + and E– plants with soil AFP were independent of the plant genotype (Fig. 4a,b). The results indicated that in the limiting oxygen range, the activities of both genotypes' antioxidant enzymes were significantly greater than in the E– plants than in E + plants (Fig. 4a,b). The greater antioxidant enzyme activity in the E– tall fescues may show that they experienced more oxidative stress under oxygen-limited conditions. Thus, it is likely that the fungal endophyte reduced the flooding-induced oxidative stress by preventing ROS's over-accumulation in the plant cells. Alternatively, it could also be expected that endophyte coped with oxidative stress through the formation of low molecular mass antioxidants (e.g., ascorbate, glutathione, phenolic compounds, and alkaloids). Specifically, endophytes usually stimulate the host grass to produce a range of alkaloids (Bush et al. 1997) and other secondary metabolites such as phenolic compounds (Ju et al. 1998) under abiotic stresses. The enhanced production of antioxidants (alkaloids, phenolics, mannitol) by endophyte-infected grasses may also be considered as a drought tolerance mechanism to protect meristems and cell membrane functions from the detrimental effects of reactive oxygen species (ROS) (Qawasmeh et al. 2012; White Jr and Torres 2010). Therefore, the production of low molecular mass antioxidants could be a possible reason for a better performance of E + plants under oxygen-limited conditions, which needs to be investigated in future studies.

In this study, the endophyte ameliorated oxidative stress by reducing adverse effects of oxygen deficiency on plant performance, leaf chlorophyll, and water content. Thus, the E + plants experienced less oxidative stress and produced lower antioxidant enzymes. The negative and significant ($p < 0.01$) strong

correlations between Chlo *T* and CAT and APX ($r = -0.93$ and $r = -0.92$), moderate correlations between root/shoot ratio and CAT and APX ($r = -0.69$ and $r = -0.57$) and strong correlations between RWC and CAT and APX ($r = -0.81$ and $r = -0.89$) support this conclusion.

The oxygen deficiency in the root zone often leads to an increase in the ADH activity as metabolism is shifted from aerobic to fermentative pathways (Benz et al. 2007). An increase in the ADH activity may decrease the phytotoxic effects of the accumulation of acetaldehyde or lactate under oxygen-limited conditions (Perata and Alpi 1991). It can help maintain ATP production (Dennis et al. 2000) and regulate the cytoplasmic pH in the absence of O_2 (Roberts et al. 1984).

Our findings of increased ADH activity in the oxygen-deficiency conditions (Fig. 4c) are in line with the results of previous studies (Benz et al. 2007; Luzhen et al. 2005; Yiu et al. 2011). The ADH activity varied with endophyte infection depending on plant genotype; thus, its values were higher in the E− 75B and E + 75C plants than those in the E− 75C and E + 75B under saturated conditions (Fig. 4c). Hume et al. (2016) stated that the benefits of endophyte infection for the host grass varied in different environmental conditions, grass species, and cultivars and were highly influenced by the interactions of host–endophyte genetic combinations.

A broad taxonomic survey of plants suggested that flooding-sensitive species tend to have a greater increment in ADH activity in the absence of O_2 compared to flooding-tolerant species (Luzhen et al. 2005; McManmon and Crawford 1971). However, in contrast, some studies found positive relationships between the ADH activity and flood tolerance (Mendelssohn et al. 1981; Torres and Diedenhofen 1981). Negative correlations ($p < 0.01$) were found between ADH activity and plant physiological parameters such as RWC ($r = -0.77$), Chlo *T* ($r = -0.82$), Chlo *a* ($r = -0.76$) and Chlo *fl* ($r = -0.68$), and between ADH activity and plant growth parameters such as DW_{root} ($r = -0.57$, $p < 0.01$), RV ($r = -0.38$, $p < 0.05$) and DW_{plant} ($r = -0.47$ $p < 0.05$). These relations confirmed that plants with high ADH activity under oxygen deficiency showed lower flooding tolerance than those with low ADH activity. Therefore, lower ADH activity and greater values of growth parameters (DW_{root}, RV, and DW_{plant}) in E + 75B compared to E− ones (Figs. 3 and 4c) suggested that endophyte infection might increase the tolerance of 75B plants to oxygen deficiency. In contrast, the reverse trend was observed for the 75C genotype (Figs. 3 and 4c).

Aerenchyma formation (morphological adaptation)

Besides the induction of enzymes synthesis in the ethanolic fermentation pathway, an alternative mechanism for coping with oxygen deficiency is the development of aerenchymous tissue in the root cortex, which effectively facilitates O_2 diffusion from the atmosphere through the plant body to the roots and rhizosphere (Benz et al. 2007; DeLaune and Reddy 2008; Evans 2004). In this study, aerenchyma was extended in the roots of both tall fescue genotypes (Fig. 5), facilitating oxygen delivery to the root zone. Positive values of root zone Eh (Fig. 1) confirmed that tall fescue oxidized the root zone through aerenchyma formation. The percentages of root cross-section area occupied by aerenchyma in the E− plants were greater than those in the E + plants, possibly indicating lower stress sensed by endophyte-infected genotypes. Generally, the highest aerenchyma percentage was observed in the E−75C plant (Fig. 5i).

Aerenchyma is found in many crop species, including barley (Zhang et al. 2015), rice (Colmer and Pedersen 2008; Justin and Armstrong 1991), and maize (He et al. 1996). In some plants, low soil oxygen is needed for aerenchyma formation, as seen in this study in tall fescue, whereas the development of these systems in many species such as rice does not require oxygen stress. In rice, aerenchyma develops under well-aerated conditions, while hypoxic conditions further enhance the process (DeLaune and Reddy 2008). The aerenchyma allows exchanging gases between the atmosphere and soil through the plant. Beside delivering oxygen to the root tip and to the root zone and allowing aerobic respiration, it also vents harmful gases (such as carbon dioxide, ethylene and methane) from the root and soil to atmosphere (DeLaune and Reddy 2008; Shannon et al. 1996). Formation of aerenchyma and oxygenation of the root zone minimize the harmful effects of anaerobic conditions and reduce compounds on roots and also reduce the oxygen demand by the removal of some cortical cells (Evans 2004).

Conclusions

1) Lack of oxygen slightly decreased the plant growth and chlorophyll in the tall fescue. This suggests that regardless of the endophyte symbioses, both studied genotypes of tall fescue (75C and 75B) must have involved adaptive mechanisms to oxygen-limited conditions.

2) When compared with the E− plants, the E + plants showed higher shoot and root development, more leaf chlorophyll, and significantly lower CAT and APX activities under oxygen-limited conditions. Therefore, endophyte presence probably decreases the flooding-induced oxidative stress and prevents ROS's over-accumulation in plant cells.

3) The mechanisms of adaptation and tolerance to oxygen deficiency were partially different between the E + and E− plants and the two tested genotypes. The E− plants coped with the lack of oxygen by forming adventitious roots and aerenchyma (morphological adaptation) and increased activity of ADH (fermentation pathway, metabolic adaptation). In contrast, no adventitious root was observed in the E + plants, and they had lower levels of ADH activity and aerenchyma area under anoxic conditions than the E− plants. This could also be another piece of evidence that *Epichloe* endophyte may decrease flooding-induced oxidative stress and accumulation of ROS content. A detailed anatomical and physiological study of adventitious roots in this plant under oxygen-limited conditions is recommended.

4) The presence of endophyte improved the performance of genotype 75B under anaerobic conditions, while endophyte had an adverse effect on the performance of genotype 75C. The E + 75B showed the highest tolerance to oxygen-limited conditions among the tested genotypes, followed by E− 75B and E− 75C (both were similar) and E + 75C as the lowest flooding-tolerant one. Generally, mechanisms of adaptation and tolerance to oxygen deficiency of endophyte-infected genotypes of tall fescues are not yet understood. In this study, we illustrated that some combinations of *Epichloë* and tall fescue genotypes like E + 75B + and their offspring could be more considered for cultivation under the waterlogged area of the world.

Abbreviations

Property	Definition	Unit
ADH	Alcohol dehydrogenase	U g FW^{-1}
AFP	Air-filled porosity	m^3 m^{-3}
APX	Ascorbate peroxidase	U g FW^{-1}
CAT	Catalase	U g FW^{-1}
Carot	Carotenoids	mg g FW^{-1}
Chlo *a*	Chlorophyll *a*	mg g FW^{-1}
Chlo *b*	Chlorophyll *b*	mg g FW^{-1}
Chlo *T*	Total chlorophyll	mg g FW^{-1}
Chlo *fl*	Chlorophyll fluorescence	–
D_p	Soil-gas diffusion coefficient	cm^2 s^{-1}
DW_{shoot}	Shoot dry weight	g per pot
DW_{root}	Root dry weight	g per pot
DW_{plant}	Plant dry weight	g per pot
Eh	Redox potential	mV
E+	Endophyte-infected plants	–
E–	Endophyte-free plants	–
FW_{plant}	Plant fresh weight	g per pot
h	Absolute of matric potential	hPa
k_a	Soil air permeability	μm^2
LWP	Leaf water potential	MPa
RV	Root volume	cm^3 per pot
RWC	Relative water content	%

Declarations

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Figures

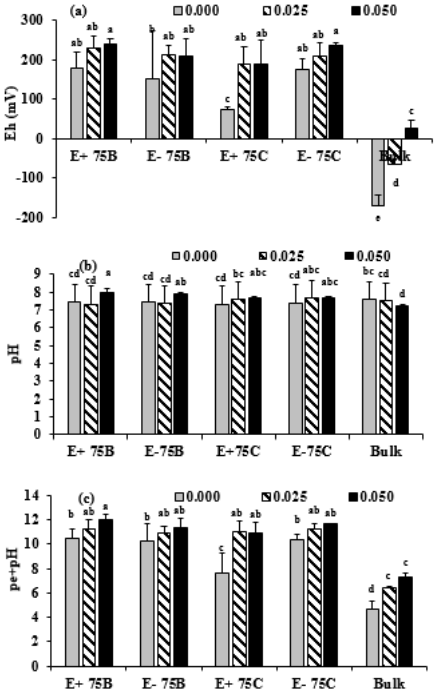

Figure 1

The effect of varying soil air-filled porosities (AFP) on redox potential (Eh) (a), pH (b) and pe+pH (c) of the bulk and the root zone soils of 75B and 75C genotypes for both cases of endophyte-infected (E+) and endophyte-free (E−). The vertical bars indicate means ± standard deviations (n=3 replications). The letter on the bars indicates a significant difference among different treatments (LSD, $p < 0.05$).

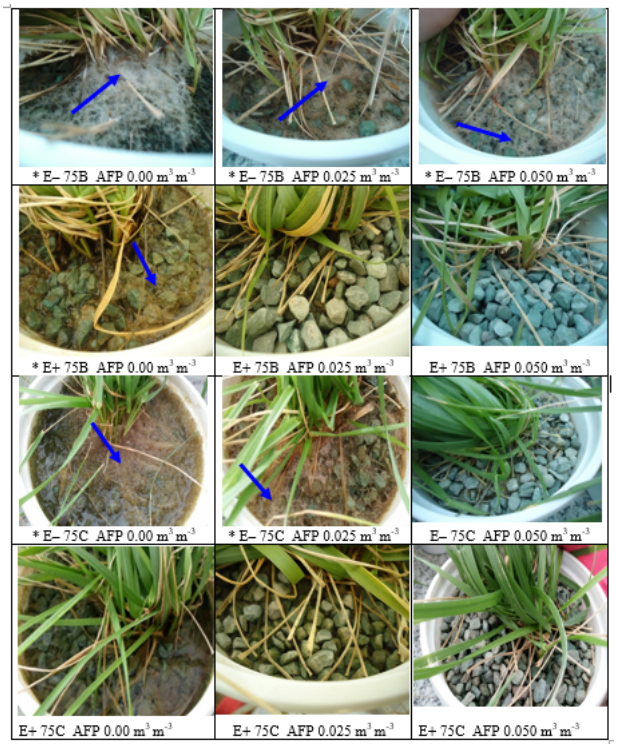

Figure 2

Some exemplary photographs of adventitious root formation (shown by arrows) in 75B and 75C genotypes of endophyte-infected (E+) and endophyte-free (E−) tall fescue plants at three levels of soil air-filled porosity (AFP values of 0.00, 0.025 and 0.050 m3 m-3). * indicates here the treatments that showed formation of adventitious roots on the soil surface.

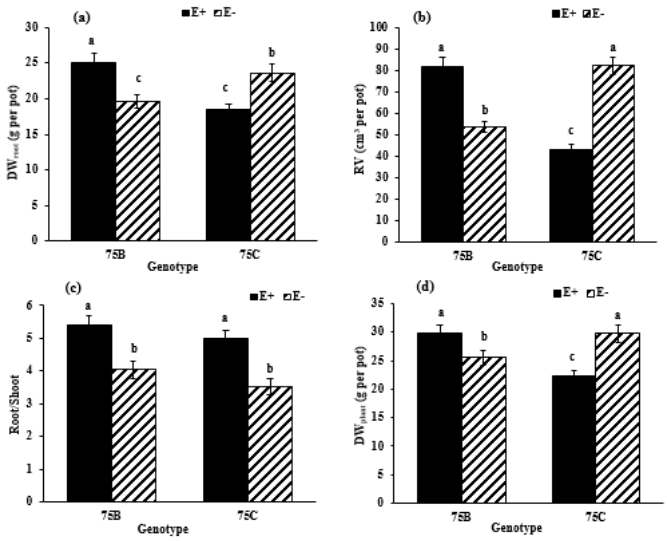

Figure 3

Mean comparisons of interaction effects of endophyte status (E+ and E−) and tall fescue genotype (75B and 75C) on growth parameters: (a) dry weight of root (DWroot), (b) root volume (RV), (c) ratio of dry weights of root to shoot (Root/Shoot), and (d) dry weight of plant (DWplant); Bars with different letters indicate significant differences (LSD, $p < 0.05$); The vertical bars indicate means ± standard deviations.

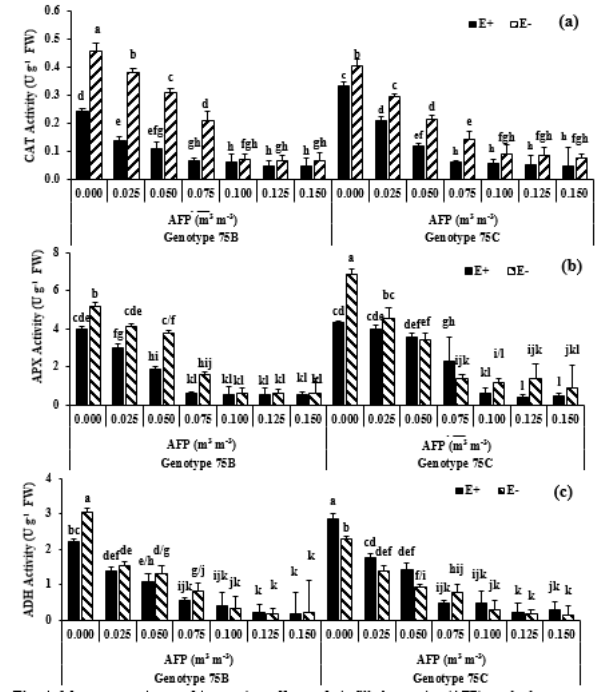

Figure 4

Mean comparisons of interaction effects of air-filled porosity (AFP), endophyte status (E+ and E−), and genotype (75B, 75C) on enzymes activities: (a) catalase (CAT), (b) ascorbate peroxidase, and (c) alcohol dehydrogenase (ADH); Bars with different letters indicate significant differences (LSD, $p < 0.05$); The vertical bars indicate means ± standard deviations.

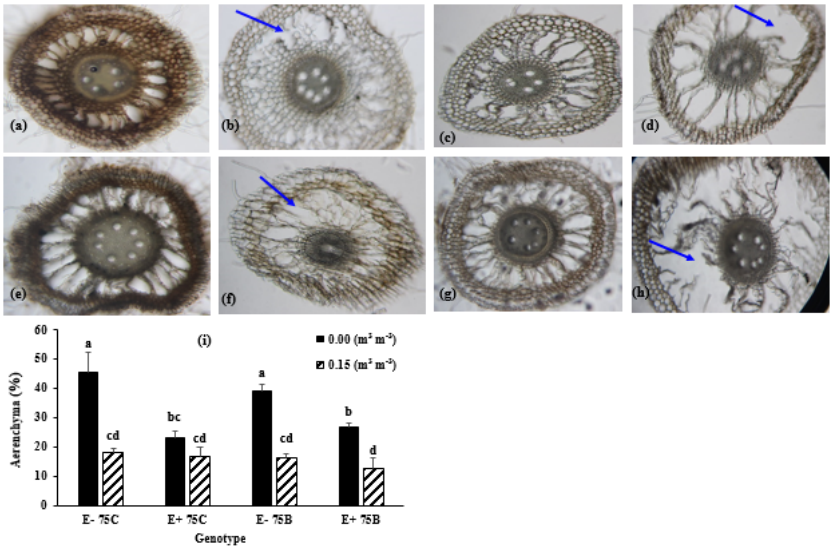

Figure 5

Anatomy of apical region of tall fescue roots of the genotype 75B: a) endophyte-infected (E+) at soil air-filled porosity (AFP) of 0.150 m3 m-3, b) E+ at AFP=0.0, c) endophyte-free (E−) at AFP=0.150 m3 m-3, and d) E− at AFP=0.0 and genotype 75C: e) E+ at AFP=0.150 m3 m-3, f) E+ at AFP=0.0, g) E− at AFP=0.150 m3 m-3, and h) E− at AFP=0.0. (i): The effect soil air-filled porosity (AFP) on the percentage of aerenchyma formation in the roots of 75B and 75C genotypes for both cases of endophyte-infected (E+) and endophyte-free (E−). Bars with different letters indicate significant differences (LSD, $p < 0.05$); The vertical bars indicate means ± standard deviations.