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Comparative physiological study of endophyte-infected and non-infected genotypes of *Lolium perenne* under drought stress

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Abstract

To evaluate the effect of drought stress on some physiological characteristics of six endophyte-infected (E+) genotypes and four endophyte-free (E-) genotypes of *Lolium perenne*, an experiment was carried out within a completely randomized design in three replications. In pot culture, one- month stress conditions for E+ and E- genotypes of *L. perenne* were handled by limiting irrigation to fulfill 20% field capacity of the soil. The physiological characteristics of E+ and E- genotypes of *L. perenne* indicated a better resistance of E+ genotypes under drought stress in terms of higher biomass and relative water content, deeper root system, and greater osmolytes accumulation and antioxidant potential. It is concluded that the osmotic adjustment (higher content of proline and total carbohydrate), lower oxidative biomarkers (H_2O_2 and MDA content), higher enzymatic and nonenzymatic components (phenolic, GSH), and induction of stress hormone (Abscisic acid (ABA)) are key protective mechanisms in E+ genotypes under drought stress. In contrast, E- genotypes of *Lolium* had some compliant mechanisms to cope with drought stress, including enhanced antioxidant activity, presented lower lipid peroxidation, improved osmolyte accumulation, induction of ABA hormone and up-regulation of *Lptip1;1* and *Lptip1;2*; aquaporin genes. It seems that E- genotypes apply the defensive antioxidant mechanism more than E+ genotypes and use more ABA as a critical component of the stress acclimation mechanism in drought conditions.

Keywords Abscisic acid, Antioxidants, Drought stress, Endophyte fungus, *Lolium perenne*

Introduction

Grasses adapted to cold climates are one of the most valuable species from an economic and environmental point of view because they play a key role in carbon fixation and are healthy fodder for livestock consumption [1]. Many cool-season grasses are hosts to fungal endophyte species, among which *Epichloë festucae var. lolii* is commonly found in *Lolium perenne* L. (perennial ryegrass). The fungus colonizes the intercellular spaces of the leaf sheaths, stems, and flowers of host grasses to become disseminated through the seeds [2]. Numerous studies have reported that symbiotic associations with

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endophytes can play a significant role in grass adaptation to abiotic and biotic stress [3]. The symbiotic relationship confers abiotic stress tolerance by activating the host's stress-response systems promptly upon stress exposure, thereby avoiding or mitigating the impact [4]. Endophytes enhance drought tolerance in their host plants by growth and productivity improvement, altering root structures, accumulating compatible solutes, adjusting water relations and photosynthesis, improving phytohormone regulation and antioxidative capacity, and regulation of drought-related genes [5–7]. All the mentioned morphophysiological alterations above are related to a highly regulated dynamic response at the molecular and cellular level, which the plant's response depends on the stress intensity and the genotype [8]. Nevertheless, the mechanisms activated by endophytic fungi in the mitigation of drought stress are an open topic of study [9, 10].

Perennial ryegrass is one of the most cultivated species deemed suitable for agriculture [11], as it combines fodder yields and nutritive value. Perennial ryegrass is a drought-sensitive grass species; however, significant genotypic variation in response to drought stress has been demonstrated [12]. Drought stress is a major abiotic factor that limits plant growth and crop productivity worldwide [13], seriously jeopardising plant production in 25% of the world's land. In Iran, drought stress is the primary environmental challenge limiting agricultural production in the semi-dry and rain-fed areas. Loca et al. 2019 reviewed that drought stress negatively disturb water relations, carbohydrate metabolism, respiration, and induces the generation of ROS, lipid peroxidation and denaturing of proteins in cool-season grass species [14]. The *Epichloe-Lolium* association is generally considered to confer drought tolerance to *L. perenne* as supported by many studies [15–17]. Notably this effect varied depending on many factors such as the endophyte strain, the host genotype, and the type of stress [18, 19]. However, there are some reports demonstrating no evidence for improved drought tolerance by endophyte infection [20–22]. In the most recent meta-analysis conducted by Dastogeer (2018), fungal endophytes influence plant performance differently based on the context [23]. In drought conditions, endophytic effects in plants were more pronounced than in plants grown under normal irrigation. The interaction outcome between a plant and a fungus is determined by their specific identities.

The objective of the present study was to examine the effects of endophytic fungi on the ability of *L. perenne* genotypes to recover from drought stress. The working hypothesis was that endophytes would improve host growth during drought stress, but the extent of improvement would depend on the host genotype. Therefore, in the present study, *Lolium* biomass, relative water content, osmo-protective molecules, abscisic acid concentration, glutathione content, antioxidant enzymes activities, and gene expression of aquaporins among ten genotypic combinations (6 E + genotypes and 4 E- genotypes) were compared under drought stress.

Material and methods

Plant material of this experiment consisted of six E + genotypes (C8, S10, S9, C10, C9 and C6) and two E- genotypes (C7, S3, Vigor and Speedy) of *L. perenne*, whose parents were used in previous research and collected from different regions of Iran, especially the North and Northeast [24]. Two commercial E- genotypes including Vigor and Speedy (Burenbrug Company, Netherlands) were also used as control in the experiment. Except two commercially available genotypes of Vigor and Speedy, seeds of other genotypes of perennial ryegrass were collected previously from Jangal-e Abr (Cloud Forest), Golestan, located at 36.7531° N, 55.0317° E, by Torkian et al. [24]. Eight native genotypes along with two commercial genotypes were surveyed in a greenhouse pot culture in compliance with a completely randomized design with three repetitions. Greenhouse conditions included a 16 h/8 h light/dark photoperiod at a temperature range of 18.3 to 25.5 °C.

The uniform and healthy tillers were cultivated in pots (size: 18*16 cm) containing mixture of sand and soil in the ratio of 1:3 (v/v) and were placed in greenhouse conditions. The results of soil chemical analysis were as pH: 7.78, E.C.: 1.34 dS m^{-1}, and available organic matter, nitrogen, phosphorus and potassium were 0.45%, 0.043%, 8.9 and 252 mg/kg, respectively. An irrigation program was carried out every day for a week and then based on the plant's needs. We determined the endophyte status in the leaf sheaths of all plants by Saha et al. [25] method before and at the end of the experiment. Following eight weeks (60 days) of growth, half of the pots were subjected to a dry-down, and the remaining pots were irrigated to approximately preserve their container capacity. The plant was subjected to drought stress treatment through a controlled dry down process, where soil moisture was gradually decreased to 20% of the container's capacity and maintained within this range for a period of 30 days. Soil moisture content was determined using a Theta Probe soil moisture sensor (AT Delta-T Devices SM300, from Cambridge, England). Pots receiving drought treatment were subsequently rehydrated to 90% of their container capacity on day 90 and maintained within this range for a period of 1 month (Fig. 1).

Physiological parameters were measured under drought stress and control conditions. Fresh leaf tissue samples for biochemical analysis were collected at one week after stress, then sealed in plastic bags, frozen with liquid nitrogen, and stored at − 80 °C for examination of H_2O_2, MDA, antioxidant enzymes, and ABA

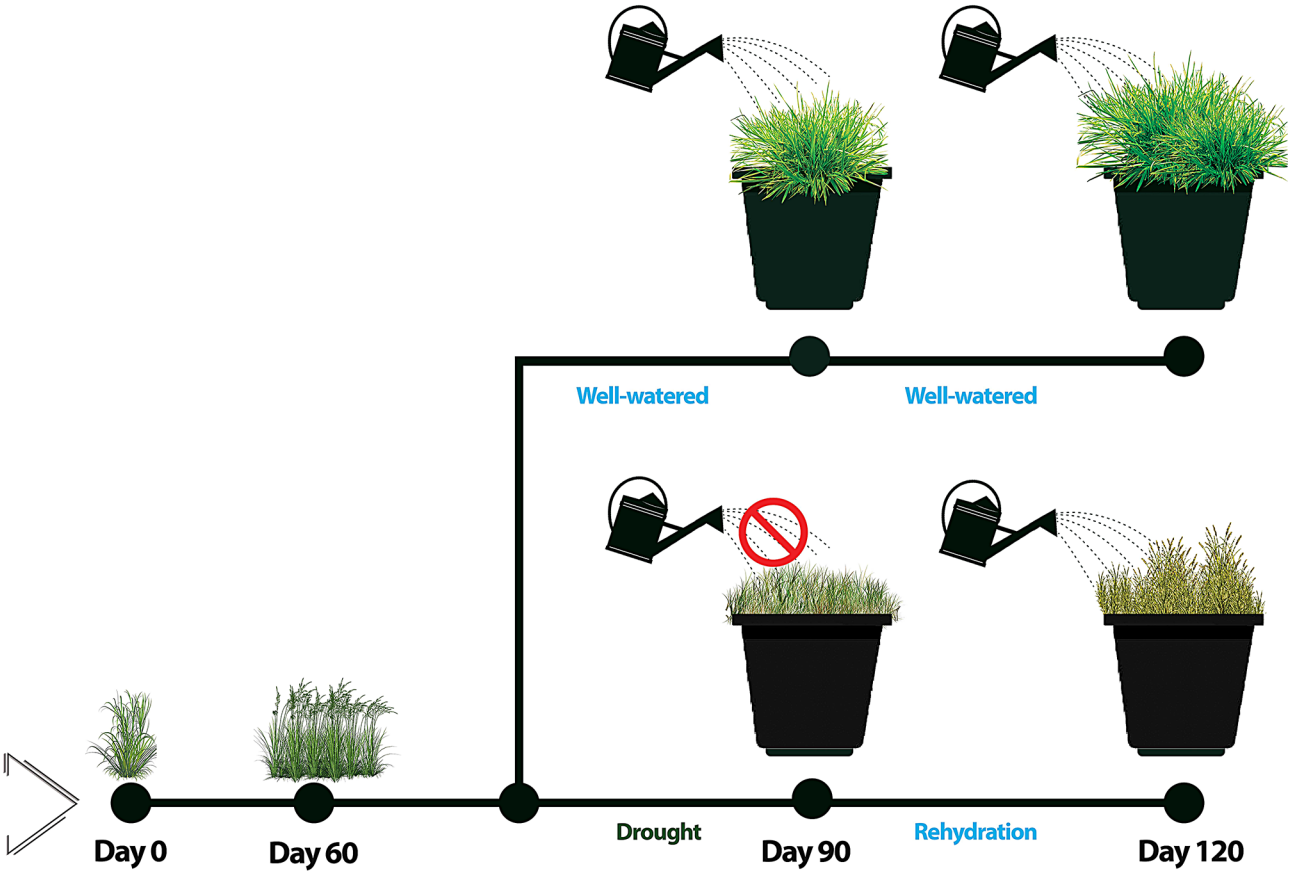

Fig. 1 The timeline for endophyte infected (E+) and non-infected (E-) genotypes of *Lolium perenn* under well-watered, drought and rehydration conditions

levels, as well as to analyze gene expression levels. The dry weights of shoots and roots, as well as the relative water content (RWC), proline, total phenolic, total carbohydrate, and glutathione contents, were measured at the end of drought stress period. The harvested material were washed with distilled water and dried on filter paper at the end of experiment. To prevent the loss of fine roots when washing the plant roots, a 1 mm mesh was used. The leaves and roots were separated for each plant. The leaves and roots were dried at 65 °C for 48 h. The dry weights were then calculated. After one month of rehydrarion, the green and dried parts of the plants were separated and weighed.

RWC measurment

RWC of leaves were determined using the method of Barrs and Weatherley [26].

Proline content

The free proline content in 0.1 g of fresh leaf samples was evaluated as described by Troll and Lindsley [27].

H_2O_2 assay

H_2O_2 concentration was extracted and quantified after reaction with potassium iodide (KI) according to Alexieva et al. [28] method.

MDA assay

The level of MDA in the leaves was calculated using the thiobarbituric acid (TBA) test [29] to survey the membrane damage.

Antioxidant enzymes and glutathione (GSH) assays

Catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione S-transferase (GST) enzymes activities were spectrophotometrically detected in leaf tissue extracts as explained in our previous work [30]. The GSH content of the leaves was measured based on the method of May and Leaver [31].

Measurement of soluble carbohydrate content

Freeze-dried leaves (0.1 g) were used to determine the concentration of soluble carbohydrates. It was extracted in 5 ml of 80% ethanol (v/v). After boiling the extracts and centrifuging with anthrone reagent, the absorption

of the samples was read at 625 nm [32]. The soluble carbohydrate content was expressed as mg/g FW glucose equivalent using the standard curve.

Determination of total phenolic compounds

Total phenolic compounds were evaluated according to Singleton and Rossi [33] procedure.

Determination of ABA

We extracted ABA according to the procedure advanced by Kelen et al. [34], with some modifications. ABA was detected at 265 nm using High-performance liquid chromatography technique as explained in our previous work [30].

Quantitative real-time PCR

Extraction of total RNA from 100 mg of fresh leaves of ten genotypes of *Lolium* was performed using the DENA zist ASIA kit (DENAzist Asia Co., # S-1010-1, Iran). First strand cDNA synthesis was performed using PrimeScript RT Enzyme Mix I (PrimeScript TM RT reagent Kit, Takara Company Inc., Otsu, Japan). The primers for elongation factor 1-alpha 1 (*eEF1A*): (*eEF1A*-F 5'- CCG TTTTGTCGAGTTTGGT-3', *eEF1A*-R 5'- AGCAACTG TAACCGAACATAGC-3'), *L. perenne* tonoplast proteins 1;1 (*Lptip1;1*): (*Lptip1;1*-F 5'- GCGGCAACATCAGCCT CCTCA-3', *Lptip1;1*-R 5'- TCATGACGATCTCGAACA CC-3'), and *L. perenne* tonoplast proteins 1;2 (*Lptip1;2*): (*Lptip1;2* -F 5- GCGGCAACATCACCCTCTTCC-3, *Lptip1;2* -R 5- TCATGACGATCTCCAGCACA-3) genes were taken from Salehi et al. [35]. Quantitative real-time PCR was managed by the Qiagen apparatus (Rotor-Gene, USA) using SYBR Premix Ex Taq TaKaRa (Takara Bio, Inc., Otsu, Japan). For the reactions, the thermo-cycler program was regulated to 95 °C/5 min, then 40 cycles (95°C/10 s, 61°C/20 s, 72°C/10s), and finally 95°C/15 min. The melting curve analysis was carried out for PCR reactions to confirm the specificity of the reactions. $2^{-\Delta\Delta Ct}$ method was employed to quantify the gene expression levels [36].

Statistical analysis

The ANOVA analysis was done by SAS software (version 8; SAS Institute Inc., Cary, NC, USA), and LSD post-hoc testing was applied to compare the means at ≤ 0.05. Principal component analysis (PCA) was performed using R software version 4.3.3. Heat map was generated using Srplot [37].

Results

Effects of drought stress on growth parameters of E+ and E- genotypes of *L. perenne*

Figure 1 presents the results of the statistical analyses of shoot and root dry weight and RWC of the ten genotypes of *L. perenne*. Shoot dry weights of both E+ and E- genotypes significantly decreased under drought stress ($P < 0.05$). The reduction of shoot dry weights of E- genotypes was much higher than those of E+ genotypes under drought stress (Fig. 2, A). The root dry weights of both E+ and E- genotypes increased under drought stress ($P < 0.05$). Drought stress increased root dry weight more than twice in E+ genotypes, and only 22% increase was observed in E- genotypes (Fig. 2, B). The RWC content of both E+ and E- genotypes significantly decreased under drought stress ($P < 0.05$). Moreover, under drought and control conditions, the RWC content of E+ genotypes were much higher than that of E- genotypes (Fig. 2, C).

Effects of drought stress on proline, total carbohydrate, and total phenolic contents of E+ and E- genotypes of *L. perenne*

Both E+ and E- genotypes showed increased proline contents under drought stress ($P \leq 0.05$). Although, proline content was much greater in the S10 genotype (E+) than in the other genotypes under both drought and control conditions (Fig. 3, A). The investigated genotypes presented the same responses to drought stress with respect to their total carbohydrate and total phenolic contents. According to the results shown in Fig. 3B and; 5A, total carbohydrate and total phenolic contents increased significantly under drought stress. Together, the highest total carbohydrate (4397.25 mg/gFW) and total phenolic (223.23 mg/gDW) of leaves were measured under drought stress in the E+ genotypes; C6 and S9, respectively.

Effects of drought stress on oxidative stress markers in E+ and E- genotypes of *L. perenne*

The concentrations of H_2O_2 of *Lolium* genotypes in both E+ and E- increased considerably under drought stress. The highest accumulation of H_2O_2 in leaves was obtained under stress conditions in the E- genotype (S3), with the value of 385.86 µmol/gFW. However, the lowest H_2O_2 in leaves was related to well-watered E+ genotypes, especially S10 (Fig. 4, A). Similarly, drought resulted in a considerable production of MDA in all genotypes compared to control plants (Fig. 4, B). The maximum increase in content of MDA in the leaves belonged to the E- genotype of C7 (41.32 µmol g FW) under drought stress. In contrast, the lowest value for this index was achieved in well-watered condition from E+ genotypes especially S10 (Fig. 4, B).

Effects of drought stress on antioxidant capacity (enzymatic and non-enzymatic) of E+ and E- genotypes of *L. perenne*

Both E+ and E- genotypes significantly increased the total glutathione content under drought stress ($P < 0.05$)

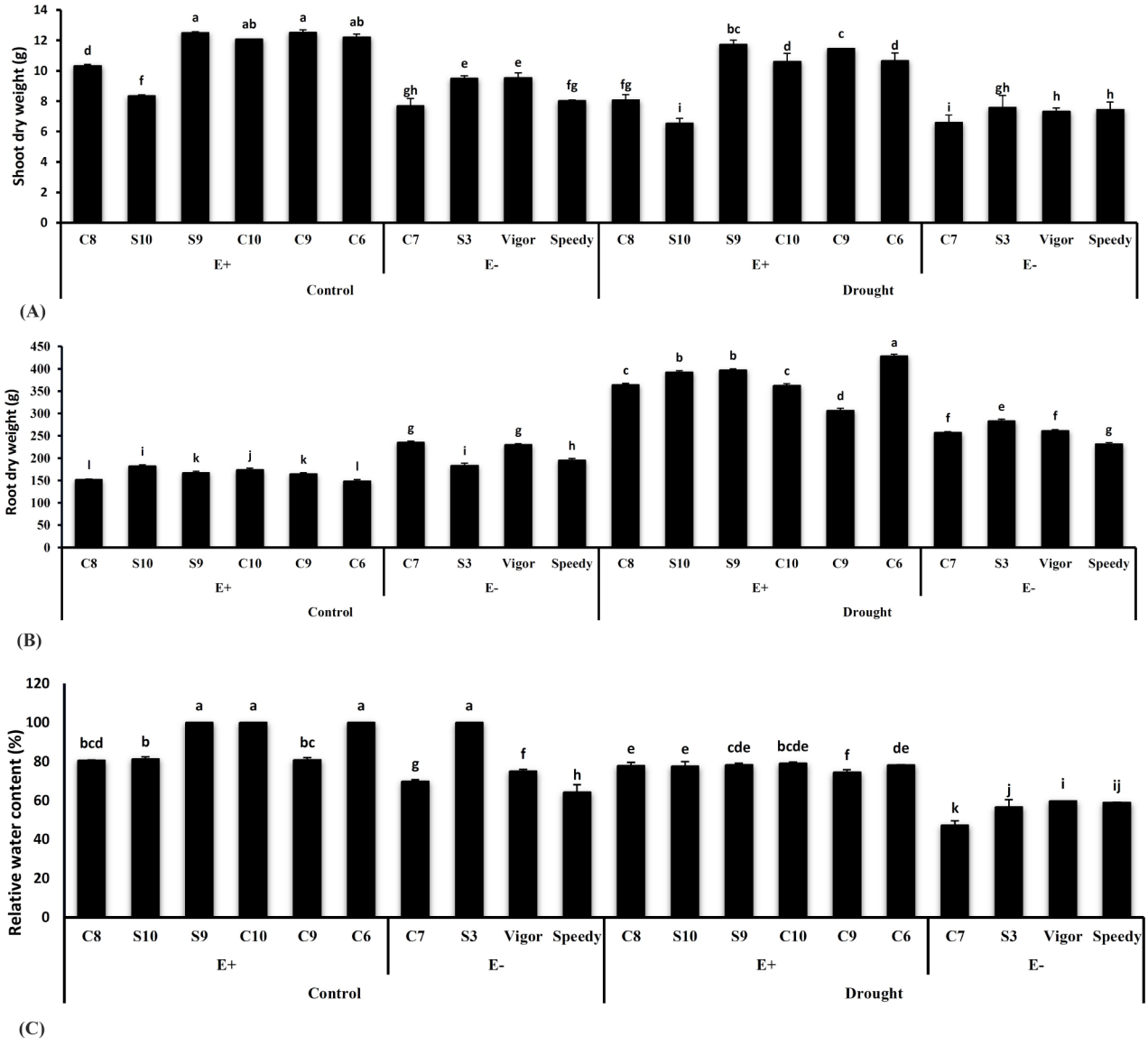

Fig. 2 Dry weights of shoots and roots (**A** and **B**) and RWC (**C**) in endophyte infected (E+) and non-infected (E-) genotypes of *Lolium* under drought stress. Different letters indicate significant differences according to LSD tests (*p*-value ≤ 0.05)

(Fig. 5, B). Moreover, the total glutathione content of E+ genotypes was much higher than that of E- genotypes under both drought and control conditions. The highest content of total glutathione in leaves was obtained under stress condition in the E+ genotype (C6), with the value of 7.22 µg/g FW. According to the results shown in the figure C, GSH: GSSG ratio in both E+ and E- genotypes decreased significantly under drought stress. Together, the highest GSH: GSSG ratio in leaves was measured in the E + genotype S9 under both drought and control conditions (Fig. 5, C).

Compared to the control conditions, antioxidant enzymes (including CAT, SOD, GPX, APX, GR, and GST) activity in leaves was significantly enhanced in all E+ and E- genotypes exposed to drought stress. The highest CAT, SOD, GP, APX, GR, and GST activities in leaves were measured in the E+ genotypes of S9 (184.6 U/g FW), S9 (1.87 U/g FW), C10 (11.89 U/g FW), C9 (7.61 U/g FW), S10 (0.78 U/g FW) and C8 (0.72 U/g FW), respectively, under drought stress(Table 1).

Effects of drought stress on ABA concentration in E+ and E- genotypes of *L. perenne*

Both E+ and E- genotypes significantly increased the ABA concentration under drought stress ($P < 0.05$). ABA concentration was higher in E+ genotypes than in E- genotypes under both drought and control conditions (Fig. 6). The enhancement of ABA concentration

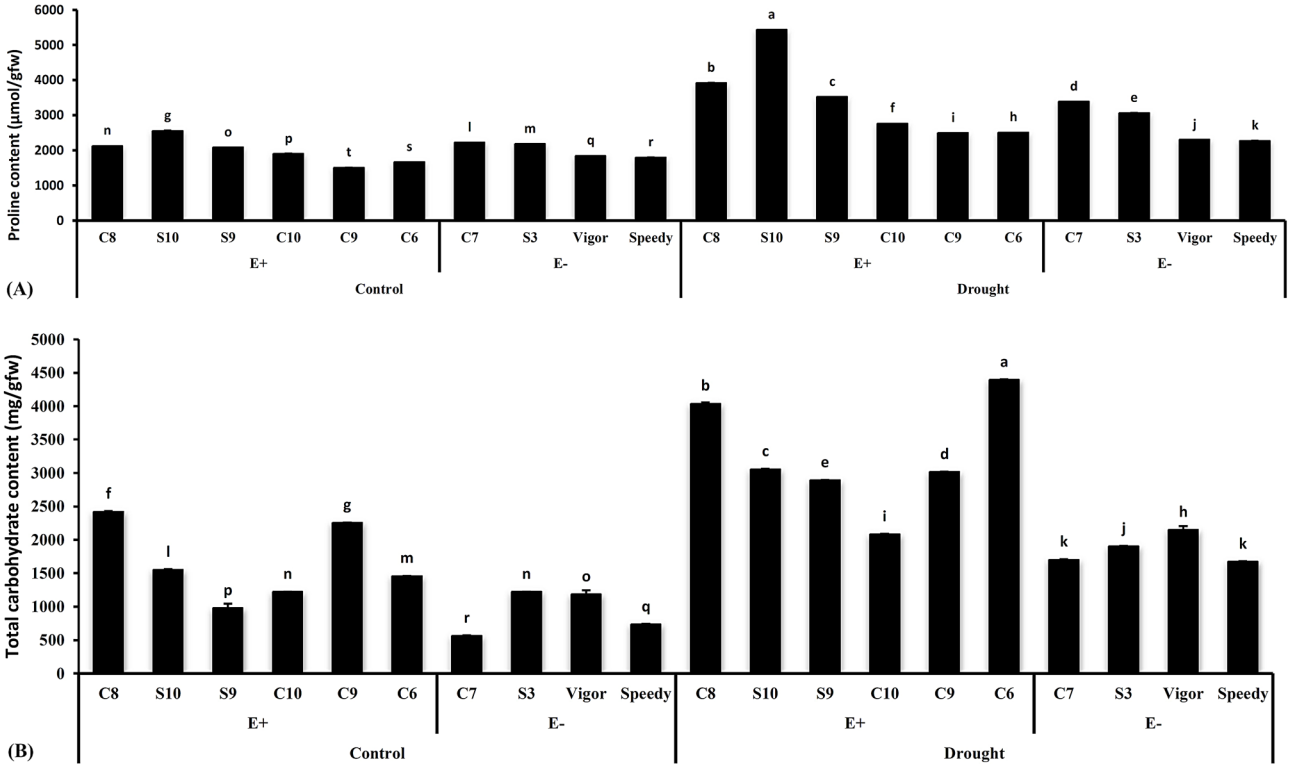

Fig. 3 Proline (**A**) and total carbohydrates (**B**) accumulation in endophyte infected (E+) and non-infected (E-) genotypes of *Lolium perenne* under drought stress. Different letters indicate significant differences according to LSD tests (*p*-value ≤ 0.05)

in E- genotypes under drought stress was much higher than that in E+ genotypes. At stress condition, the ABA concentration of C7, S3, Vigor and Speedy genotypes increased by 7.6, 5.7, 6.2 and 5.1 folds, respectively, compared to the control condition.

Effects of drought stress on the plant weight and the expression level of aquaporin genes in E+ and E- genotypes of *L. perenne*

Obviously, significant changes occurred at the expression levels of *Lptip1;1* and *Lptip1;2* genes under drought stress, but the range of induction in gene expression was highly genotype dependent. Figure 7 shows that expression levels of these two genes in E- genotypes (except Vigor genotype) significantly increased under drought stress. In the C7 genotype, the expression of two genes was significantly elevated under drought stress with the maximum expression level of 59 (for *Lptip1;1* gene) and 28 (for *Lptip1;2* gene) times higher than other genotypes. At stress condition, the expression level of *Lptip1;1* of the C9 genotype and the expression level of *Lptip1;2* in the S10 genotype increased by 1.8 and 2.1 folds respectively, compared to the control condition, although they were reduced or remained unchanged in the other E+ genotypes.

In both E+ and E- genotypes, the weight of dried parts of plants increased considerably under drought stress (Fig. 8, A). This increase in the weight of dried parts was more evident in E- genotypes. The maximum increase in the weight of dried parts belonged to the S3 genotype (74.2 g) under drought stress. On the other hand, drought stress led to the reduction of the weight of green parts in both E+ and E- genotypes (Fig. 8, B). This decrease in the weight of green parts was more evident in E- genotypes. The highest weight of green parts belonged to the S9 genotype (69.6 g and 44.8 g) under both control and drought conditions, respectively.

Multivariate data analysis

The HCA heatmap for measured traits among all treatments gave interesting results. The heatmap showed that all measured physiological and biochemical traits under the imposed treatments could be grouped into three clusters (Fig. 9). These results are in accordance with the PCA biplot (Fig. 9). Accordingly, SDW, WG and RWC were placed in a distinct cluster (A); however, the highest amount of these traits was observed in both E+ (S9, C9, C6 and C10) and E- (S3) genotypes in control condition. These traits generally decreased under drought stress conditions when considering each of the genotypes individually. Cluster B consisted of enzymatic and non-enzymatic antioxidants, oxidative stress markers, RDW, WD and ABA. These traits increased under drought stress when each genotype was evaluated separately;

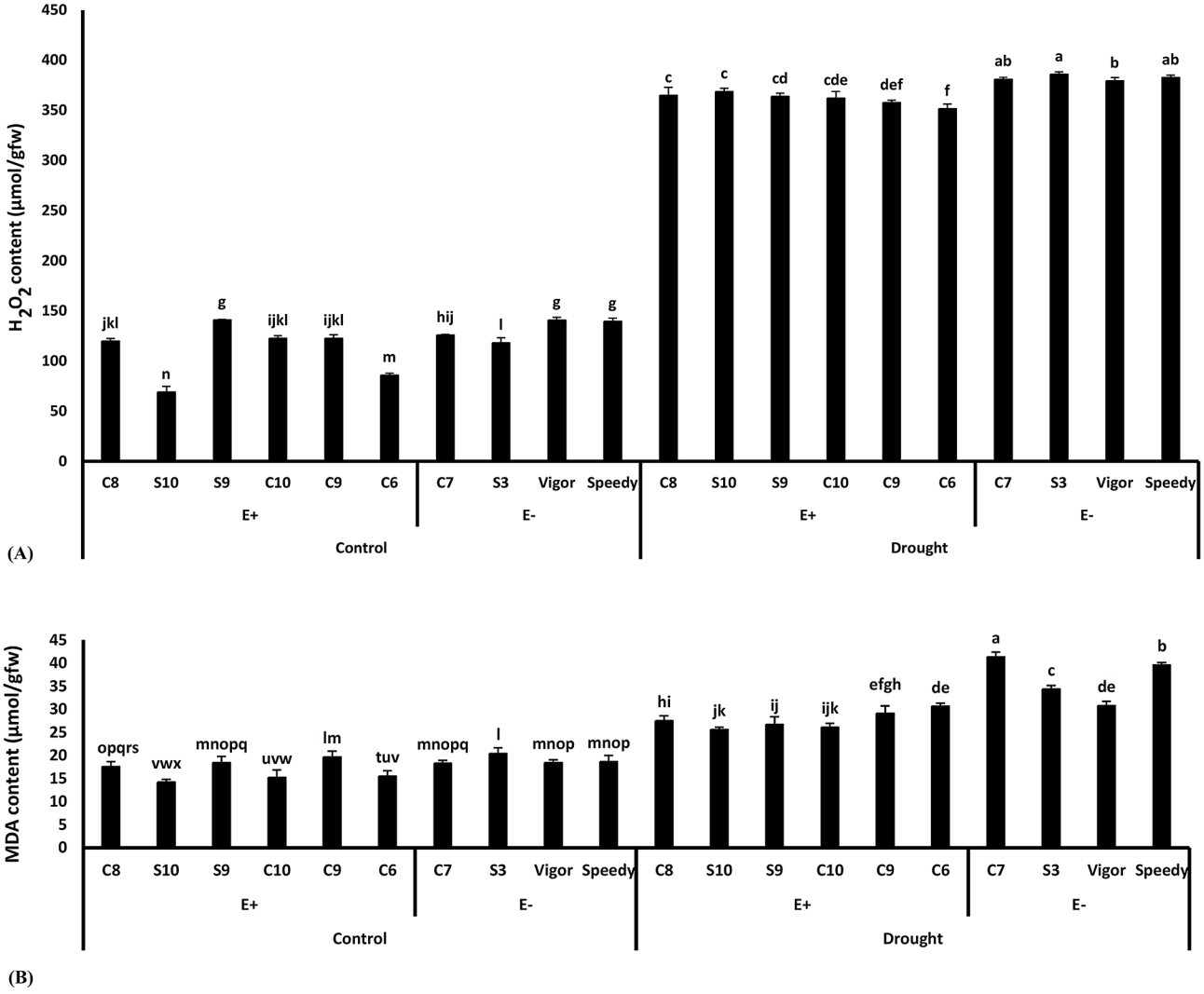

Fig. 4 Hydrogen peroxide accumulation (**A**) and lipid peroxidation (MDA) (**B**) in endophyte infected (E+) and non-infected (E-) genotypes of *Lolium perenne* under drought stress. Different letters indicate significant differences according to LSD tests (p-value ≤ 0.05)

however, in E+ genotypes, this increase was higher. The expression levels of *Lptip1;1* and *Lptip1;2* genes were placed in the cluster C; the highest amount of expression levels of these two genes was observed in E- genotypes (S3 and C7). According to the studied heatmap, it can be concluded that two E+ genotypes of *L. perenne* (i.e. S10 and C6) by having the highest values of the studied traits, could be introduced as the best genotypes to deal with drought stress.

Principal component analysis was also used to confirm the cluster analysis results (Fig. 10). This analysis put the studied treatments into three groups. Based on the available results, in the control condition, 10 genotypes of *Lolium* are placed into one group. However, the segregation of E+ and E- genotypes under drought condition occurred. As shown by cluster analysis, the E+ S10 and then C6 were considered the best genotypes under drought stress while the E- genotypes of S9 and S3 were discerned as the best ones under control conditions.

Discussion

The affirmative role of *Epichloë festucae var. lolii* fungus for improving drought tolerance in *Lolium perenne* was considered in the higher relative water content (Fig. 2C), biomass (Fig. 2A, B), weight of green parts (Fig. 8B), accumulation of osmo-protective molecules (Fig. 3) and antioxidant capacity (Table 1) in E+ genotypes under drought stress. These parameters in both E- and E+ genotypes decreased under drought stress. However, E+ genotypes had higher dry biomass, osmoprotection, and antioxidant potential than E- genotypes, stating increased drought tolerance in E+ genotypes of *Lolium*.

Multiple endophyte-mediated drought tolerance mechanisms have been implicated during drought stress in plants. Dastogeer and Wylie [9] reviewed the favourable

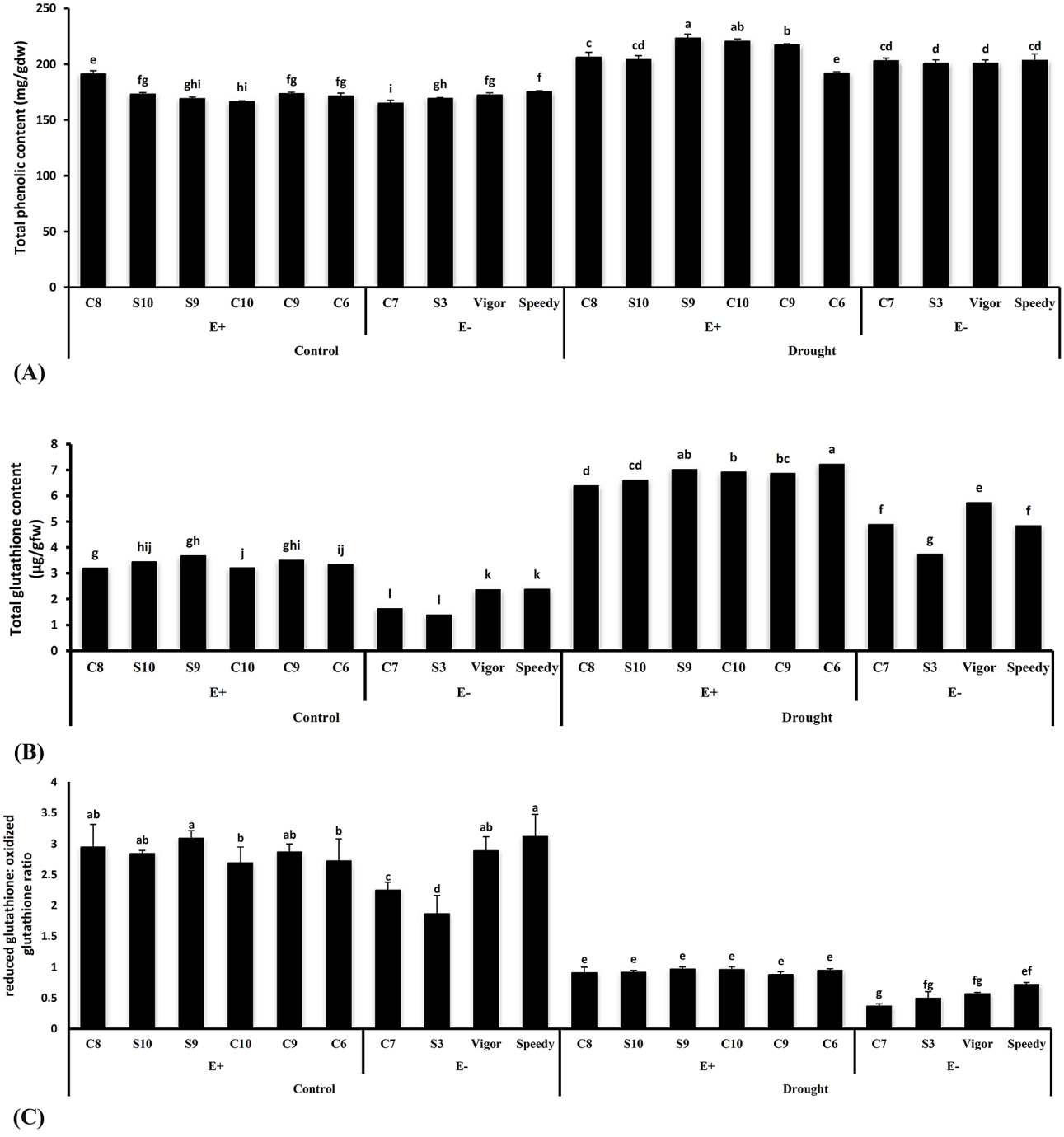

Fig. 5 Total phenolic compounds (**A**), total glotathione content (**B**) and GSH: GSSG ratio (**C**) in endophyte infected (E+) and non-infected (E-) genotypes of *Lolium perenne* under drought stress. Different letters indicate significant differences according to LSD tests (p-value ≤ 0.05)

effects of endophyte infection on the growth and water content of host plants under drought stress. E+ *Lolium* genotypes had more biomass than E- genotypes. The positive effects of this symbiotic relationship under stress are likely due to the fact that fungal endophytes produce auxin hormones that has been related to increasing the growth of plants [38].

Reports suggest that the endophytic fungi can enhance C and N accumulation, photosynthesis and plant biomass (shoot, root and total), thereby enhancing the drought stress tolerance [23, 39, 40]. However, in some cases, during drought stress, endophytes do not have beneficial effects on host growth, but they help the host plant recover quickly after re-access to water [41]. Our results revealed that compared to E- genotypes, E+ ones

Table 1 Activities of catalase (CAT), superoxide dismutase (SOD), Guaiacol peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione S-transferase (GST) enzymes in endophyte infected (E+) and non-infected (E-) genotypes of *Lolium perenne* under drought stress and control condition

Endophyte status	Genotype	Condition	CAT activity (U/gFW)	SOD activity (U/gFW)	GPX activity (U/gFW)	APX activity (U/gFW)	GR activity (U/gFW)	GST activity (U/gFW)
	C8	**Control**	120.4 ± 2.16^{ghi}	0.56 ± 0.01^{hi}	2.35 ± 0.34^{k}	$0.25 \pm 0.02^{p\text{-}t}$	0.09 ± 0.01^{jk}	$0.09 \pm 0^{n\text{-}s}$
		Drought	184.5 ± 3.85^{ab}	1.17 ± 0.05^{de}	10.95 ± 0.48^{b}	$1.15 \pm 0.17^{f\text{-}h}$	0.64 ± 0.01^{d}	0.72 ± 0.04^{a}
	S10	**Control**	$123.3 \pm 1.35^{f\text{-}h}$	0.42 ± 0.04^{kl}	1.68 ± 0.1^{lm}	$0.42 \pm 0.03^{m\text{-}o}$	$0.09 \pm 0^{j\text{-}m}$	$0.08 \pm 0^{n\text{-}t}$
		Drought	184.56 ± 3.18^{b}	1.32 ± 0.02^{b}	3.61 ± 0.11^{h}	0.91 ± 0.08^{ij}	0.78 ± 0.02^{a}	0.59 ± 0.02^{cde}
E+	**S9**	**Control**	126.06 ± 2.68^{f}	0.49 ± 0.05^{j}	2.9 ± 0.14^{ij}	0.78 ± 0.02^{jk}	0.13 ± 0.04^{i}	$0.09 \pm 0^{n\text{-}s}$
		Drought	184.86 ± 5.58^{ab}	1.87 ± 0.04^{a}	6.77 ± 0.53^{ef}	2.88 ± 0.09^{d}	0.67 ± 0.03^{cd}	0.62 ± 0.05^{cd}
	C10	**Control**	126.1 ± 5.05^{f}	0.51 ± 0^{ij}	0.23 ± 0.13^{nqr}	$0.34 \pm 0.06^{n\text{-}r}$	$0.08 \pm 0^{j\text{-}m}$	$0.08 \pm 0.01^{o\text{-}t}$
		Drought	177.98 ± 4.69^{c}	1.23 ± 0.06^{c}	11.89 ± 0.59^{a}	1.05 ± 0.19^{fi}	0.73 ± 0.03^{b}	0.61 ± 0.05^{cde}
	C9	**Control**	124.7 ± 5.16^{fg}	0.55 ± 0.03^{hi}	0.2 ± 0.09^{nqs}	$0.44 \pm 0.02^{m\text{-}o}$	$0.08 \pm 0.01^{j\text{-}n}$	0.16 ± 0.05^{m}
		Drought	187.99 ± 2.51^{a}	1.15 ± 0.05^{e}	10.87 ± 0.41^{b}	7.61 ± 0.19^{a}	0.7 ± 0.01^{bc}	0.64 ± 0.05^{bc}
	C6	**Control**	$123.8 \pm 1.52^{f\text{-}h}$	0.66 ± 0.04^{g}	0.39 ± 0.02^{no}	0.58 ± 0.01^{lm}	0.16 ± 0.05^{i}	$0.13 \pm 0.04^{k\text{-}m}$
		Drought	180.47 ± 4.51^{bc}	1.22 ± 0.03^{cd}	9.28 ± 0.19^{d}	1.68 ± 0.13^{e}	0.72 ± 0.05^{bc}	0.69 ± 0.03^{ab}
E-	**C7**	**Control**	93.26 ± 2.54^{j}	0.34 ± 0.02^{mn}	1.77 ± 0.17^{l}	0.24 ± 0.04^{prst}	$0.04 \pm 0^{n\text{-}q}$	0.03 ± 0^{tuv}
		Drought	137.56 ± 2.16^{d}	0.58 ± 0.03^{h}	2.48 ± 0.22^{jk}	0.49 ± 0.13^{mn}	0.49 ± 0^{h}	0.51 ± 0.06^{fh}
	S3	**Control**	93.23 ± 4.17^{j}	0.33 ± 0.03^{mn}	0.09 ± 0^{nqrst}	0.11 ± 0^{su}	$0.04 \pm 0^{o\text{-}q}$	0.04 ± 0^{optuv}
		Drought	$122.02 \pm 5.16^{f\text{-}h}$	1.08 ± 0^{f}	2.97 ± 0.47^{i}	0.67 ± 0.08^{kl}	0.59 ± 0.01^{ef}	$0.47 \pm 0.02^{h\text{-}k}$
	Vigor	**Control**	83.36 ± 0.97^{kl}	0.33 ± 0.03^{mn}	0.33 ± 0.04^{nq}	$0.39 \pm 0.07^{n\text{-}r}$	$0.05 \pm 0^{l\text{-}p}$	0.05 ± 0^{opqrtuv}
		Drought	126.52 ± 4.07^{f}	1.06 ± 0.04^{f}	5.89 ± 0.11^{g}	6.16 ± 0.32^{b}	0.57 ± 0.02^{eg}	0.42 ± 0.03^{il}
	Speedy	**Control**	92.6 ± 0.82^{j}	0.55 ± 0.04^{hi}	$0.45 \pm 0.04^{n\text{-}p}$	$0.34 \pm 0.01^{n\text{-}r}$	0.06 ± 0^{jlmno}	0.04 ± 0^{oqtuv}
		Drought	131.58 ± 4.26^{e}	1.06 ± 0.01^{f}	10.63 ± 0.62^{bc}	5.67 ± 0.05^{c}	0.57 ± 0.03^{eg}	0.52 ± 0.05^{fg}

In each column, means followed by the same letter(s) are not significantly different ($p < 0.05$).

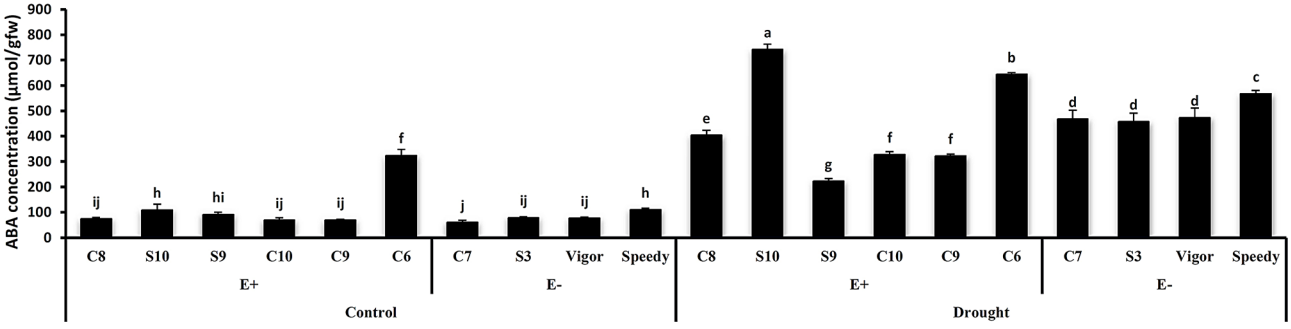

Fig. 6 ABA concentration in endophyte infected (E+) and non-infected (E-) genotypes of *Lolium perenne* under drought stress. Different letters indicate significant differences according to LSD tests (p-value ≤ 0.05)

recovered better in terms of the fresh weight of green parts upon re-watering.

One of the mechanisms of drought avoidance in E+ plants is the improvement of water absorption from the soil by a more extensive root system, which enables the plant to retain sufficient water for the above-ground organs and conserve water in plant tissues during periods of drought [42]. E+ genotypes produced more root biomass under drought stress compared to E- genotypes (Fig. 11). Some studies have found that endophytic fungi produce deeper root systems under drought stress in *Ormosia hosiei* [43]; *Quercus rubra* [44]; *Glycyrrhiza uralensis* and *Zea mays* [45, 46]. As the E+ genotypes presented deeper root system under drought indicating better water uptake from the soil, it may lead to endophyte-mediated drought tolerance.

Endophytes help plants with osmotic adjustment through the accumulation of osmo-protective molecules like proline, sugar, organic acids (e.g., malate), and inorganic ions to maintain turgor pressure, which helps the host plant to combat destruction induced by drought stress and allow cell enlargement and plant growth [47]. In this research, the accumulation of total carbohydrates and proline increased in all genotypes of *Lolium* under drought stress, but the proline content was higher in E+ genotypes than uninfected genotypes. Previous studies on tall fescue have yielded comparable results [48, 49]. Some researchers have demonstrated the effect

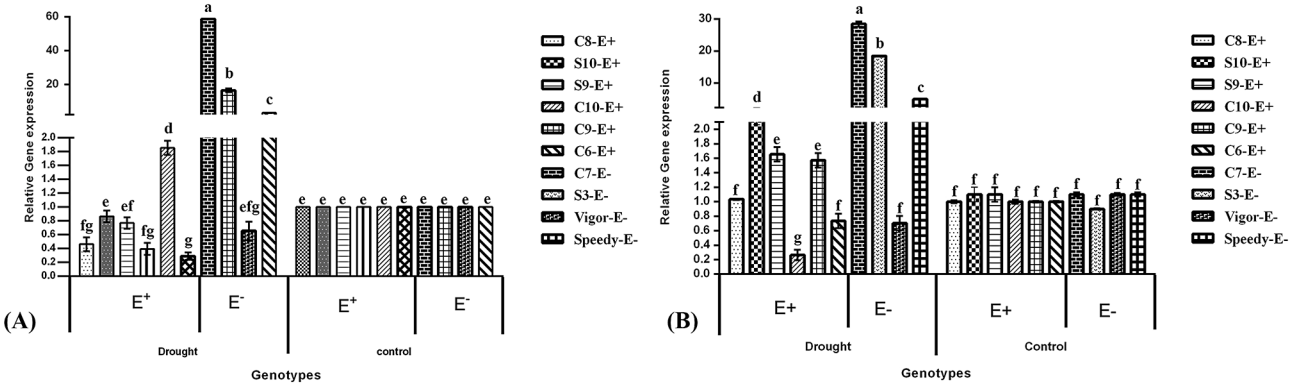

Fig. 7 The expression levels of *Lptip1;1* (**A**) and *Lptip1;2* (**B**) genes in endophyte infected (E+) and non-infected (E-) genotypes of *Lolium perenne* under drought stress. Different letters indicate significant differences according to LSD tests (p-value ≤ 0.05)

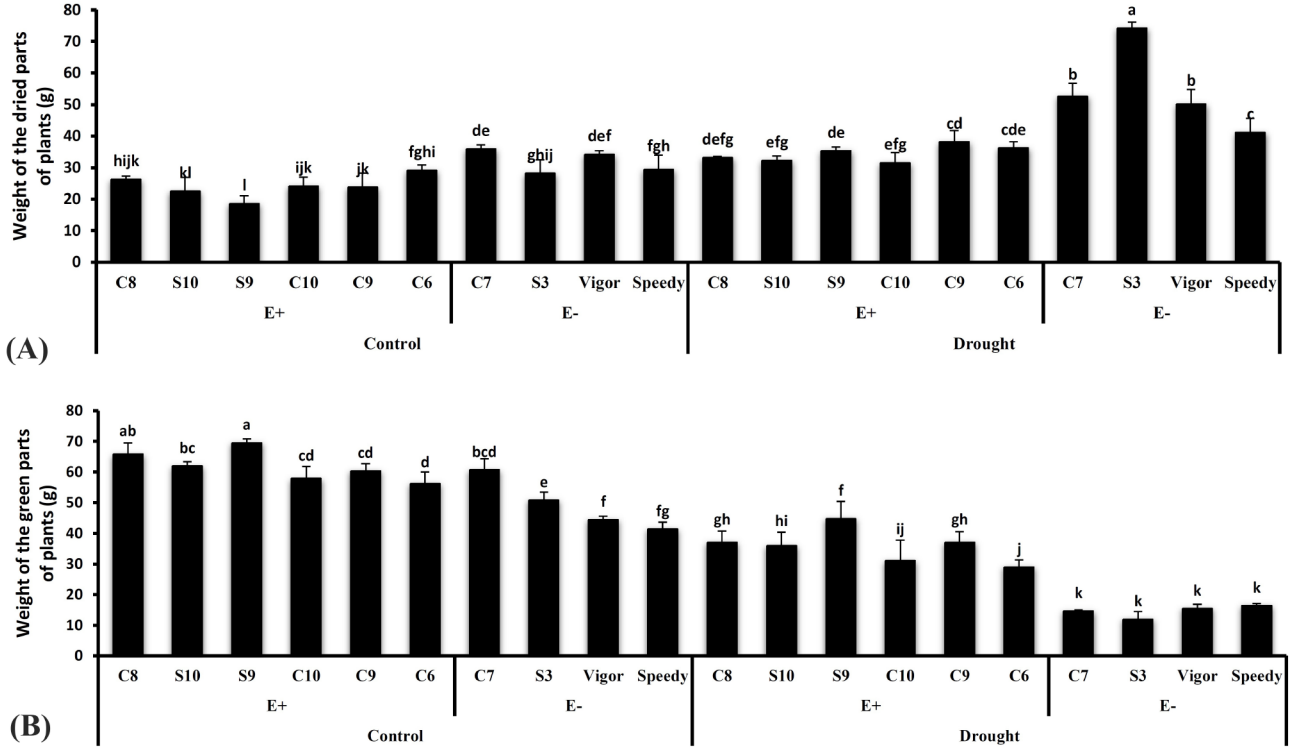

Fig. 8 The weight of dried parts (**A**) and green parts (**B**) of plants in endophyte infected (E+) and non-infected (E-) genotypes of *Lolium perenne* under drought stress. Different letters indicate significant differences according to LSD tests (p-value ≤ 0.05)

of endophytic interaction on proline content during drought stress [40, 50, 51]. Furthermore, endophyte-facilitated drought tolerance in *L. perenne* has been reported via the synthesis and redirecting of carbohydrates [52] in *Atractylodes lancea* [53], *Nicotiana benthamiana* [54], and *Triticum aestivum* [40]. These results indicate that E+ genotypes have an increased physiological response capacity to drought stress, which enables excellent osmolytes accumulation and accordingly increase osmotic adjustment by these genotypes.

Our results showed that both E+ and E- genotypes increased the production of H_2O_2 and MDA contents after exposure to drought stress. Furthermore, the changes observed in the accumulation of H_2O_2 in all genotypes matched those in their MDA content under drought stress. Recently, in agreement with this finding, Afshari et al. [55] demonstrated that all populations of *Salvia subg. Perovskia* had consistent accumulation patterns for both H_2O_2 and MDA under drought stress. In the current study, a lower accumulation of H_2O_2 and MDA was found in E+ genotypes than uninfected genotypes. Under drought stress, less level of ROS production has been reported in *Solanum lycopersicum* infected by endophyte than in control plants [56]. In a previous

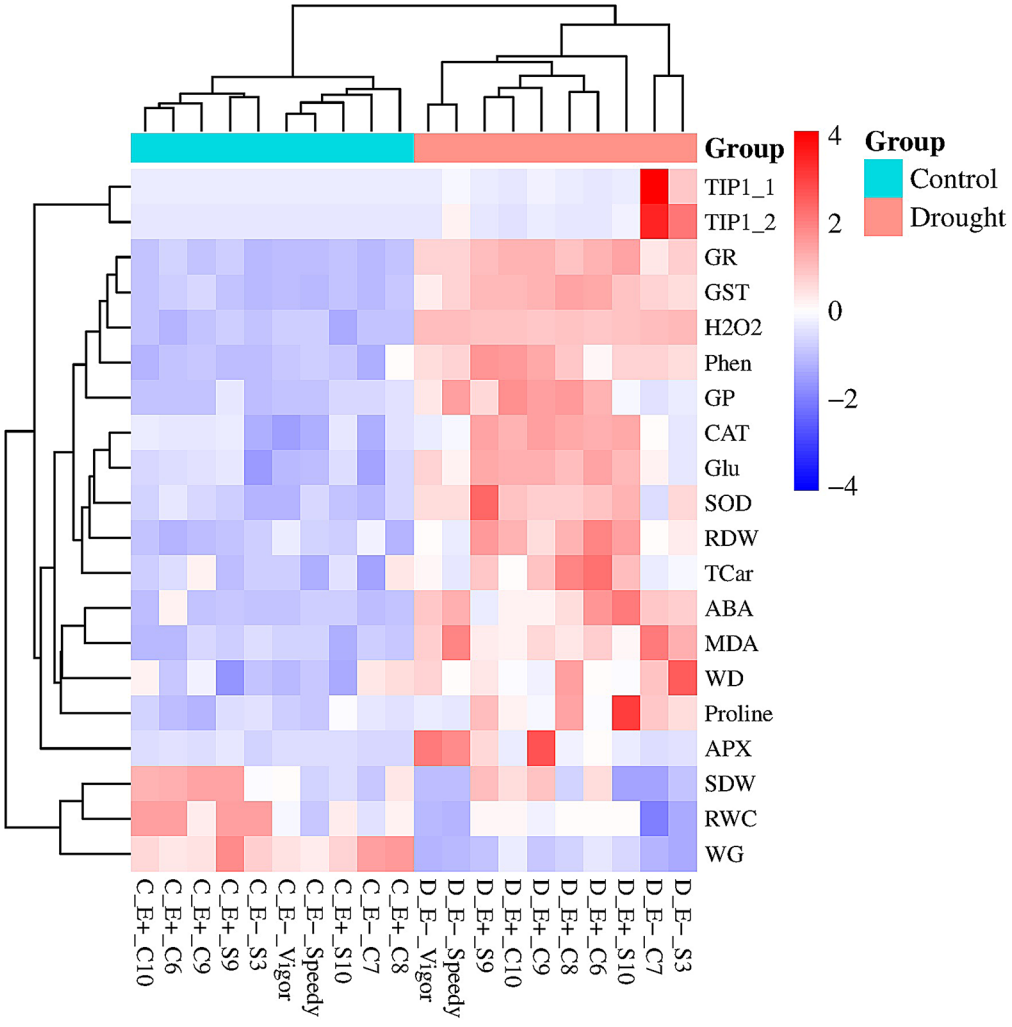

Fig. 9 The heat map-based cluster analysis of examined characteristics under drought stress and control condition in endophyte infected (E+) and non-infected (E-) genotypes of *Lolium perenne*

report, it was indicated that in comparison with E- plants of tall fescue, lower levels of electrolyte leakage, malondialdehyde content, and H_2O_2 content were observed in E+ plants during drought, re-watering, and heat stress treatment [57]. Endophyte-induced drought tolerance via lower production of H_2O_2 and MDA has been reported in *Elymus* [58], *Capsicum annuum* [59], *Z. mays* [60], and *Oryza sativa* [61] and in a new meta-analysis published by Dastogeer [23]. Therefore, the E+ genotypes of *Lolium* showed lower content of H_2O_2 under stress implying better avoidance of H_2O_2 production or scavengingH_2O_2 in these plants. Also, lower levels of MDA content were observed in the E+ genotypes exposed to drought stress indicating less lipid peroxidation resulted from ROS damage. Our results offered better ROS protection in E+ genotypes than in E- genotypes under drought stress.

Drought stress in plants results in the generation of reactive oxygen species, leading to oxidative damage. Plants are equipped with defense mechanisms involving the biosynthesis and accumulation of antioxidant compounds, mainly those related to polyphenolic antioxidants [62]. Regarding total phenolic compounds, we identified that comparing to E- genotypes, E+ genotypes particularly S10 increased higher content of total phenolic compounds under drought stress. Increment of total phenolic compounds has formerly been documented in different species under drought stress [63]. Phenolic compounds act as potent antioxidants by donating electrons, chelating transition metal ions, and slowing peroxidation through membrane rigidity [64]. Implementing these alterations could retard ROS propagation and consequently inhibit peroxidative responses [65, 66]. Up-regulation and down-regulation of enzymatic antioxidants help plants tolerate stress. Endophytes increase host plants' antioxidative capabilities, protecting them from cellular damage during stress. Our results revealed that endophyte infection enhanced antioxidant enzymes activities of leaves in all E+ genotypes of *Lolium* under

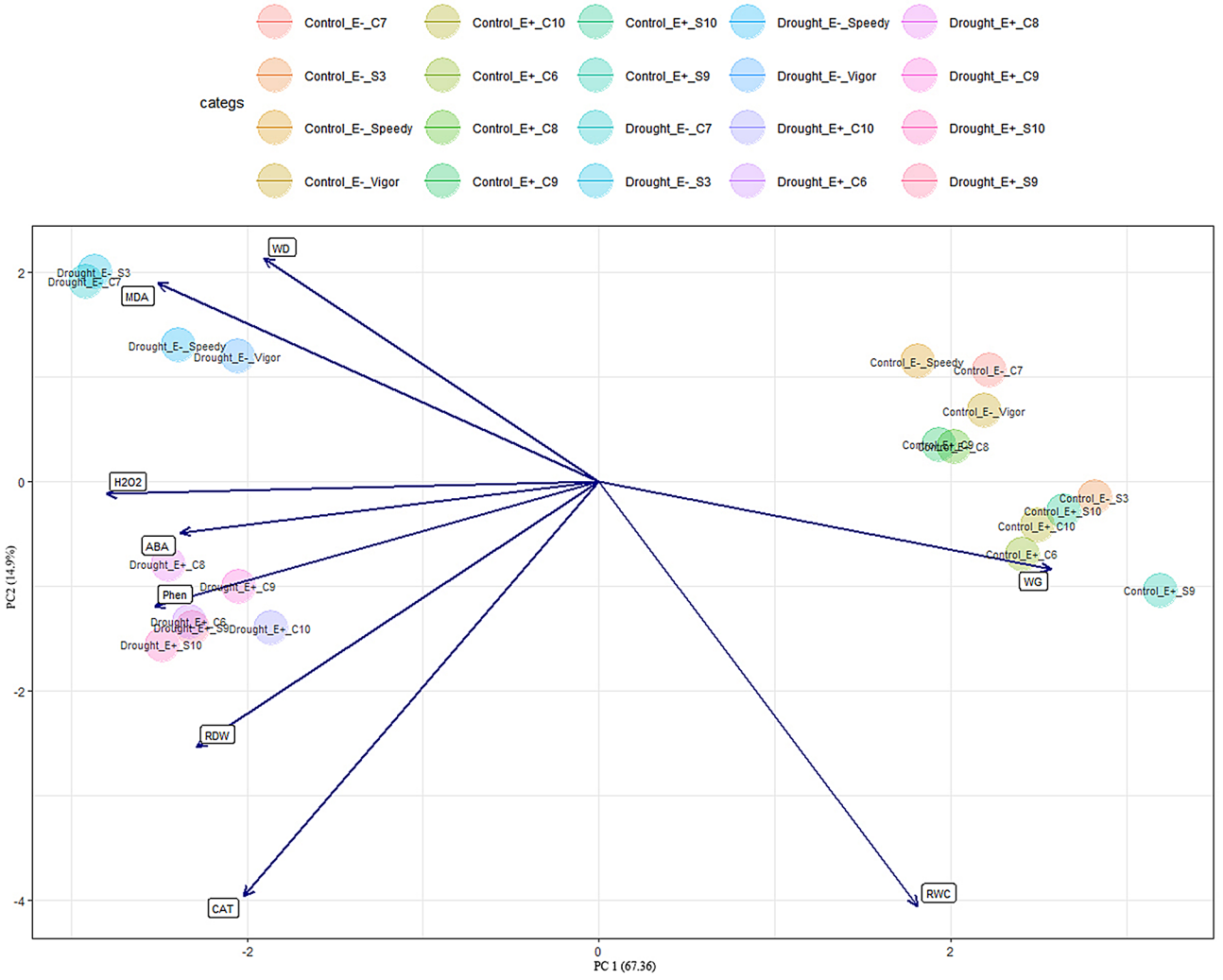

Fig. 10 The two-dimensional biplot of PCA (principal component analysis) indicates the correlation between the ten genotypes of ryegrass, drought stress, and some examined characteristics

drought stress. Different antioxidant enzymes have shown their highest values in various genotypes and it was a specific characteristic of the genotypes. This issue is also mentioned in the research of Sharma and Dubey [67]. The E+ genotypes have responded more effectively against drought-induced accumulation of ROS; the highest amount of CAT activity was measured in genotypes S9, C9, and C6, the highest amount of APX was measured in genotypes S9, SOD in C6 and S9, GP in C8, GR in S10 and C6, and GST in C8, S10, and C6. Enhancement of antioxidant defense enzymes in endophyte-colonized plants under drought stress were also found in *Triticum aestivum* [40, 68] *Populus* sp [69], and *Cucumis sativus* [70]. Ghaffari et al. [71] reported that *Piriformospora indica* upregulated glutathione S-transferase and ascorbate peroxidase isoforms in inoculated barley. According to the report of Siddiqui et al. [72], more resistant genotypes have higher enzyme activity; as a result, stabilization of membrane structure and reduction of lipid peroxidation occurs [73].

GSH content as one of the components of the non-enzymatic antioxidant system and cellular redox regulators increased in all genotypes under stress compared to the control plants. The increase of reduced glutathione was higher in the E+ genotypes, which was consistent with the increase of enzymes such as GR in these genotypes. The highest values of reduced glutathione were in S9, C6 and C10 genotypes, respectively. Along with the increase of reduced glutathione, the amount of oxidized glutathione (GSSG) also increased. The highest values were in Vigor, C7, C6, and C9 genotypes. The highest GSH: GSSG ratio was recorded in control plants and this ratio decreased in plants under stress and the lowest ratio was recorded in the genotype C7 and the highest was in genotypes C10, S9, and S10. According to the report of Hasanuzzaman et al. [74], plants with endophytic fungi

Fig. 11 Root density of non-infected (E-) genotypes; S3 (**A**), Vigor (**B**) and endophyte infected (E+) genotype; C6 (**C**) of *Lolium perenne* under drought stress

have higher antioxidant capacity and higher GSH levels. We found that the enhancement of antioxidant potential of E- genotypes was much higher than those of E+ genotypes under drought stress. Conversely to previous findings, the conducted analyses revealed that ROS scavenging enzymes were more activated in the uninfected than E+ plants. Indeed, looking at Table 1 data, it must be stated that E+ genotypes have a basal activation of such enzymes, as demonstrated by data already taken in control conditions. Under drought stress, the enzyme activities were all boosted, and there are many cases in which data are higher in E+ rather than E- genotypes. It seems that the only E- genotype in which this fact is reverted was Speedy. The upregulation of ROS-generation proteins in E- genotypes may have resulted in a higher amount of produced ROS in these genotypes than that in the E+ genotype. Therefore, to maintain homeostasis, they boosted enzyme activation.

Abscisic acid is a phytohormone involved in seed dormancy, bud growth and adaptation to environmental stresses such as drought, salinity, cold, heat and wounding [75]. Endophytic fungi, proposed by Xu et al. [57], are capable of sensing plant ABA and influencing gene transcription, protein synthesis, and degradation. Primary metabolism may alter, allowing for nutrient exchange with host cells. The secondary metabolites of fungi may also be altered for environmental adaptation. The higher content of ABA during water deficit controls plant growth by modulating root growth and altering leaf elongation and development. Abscisic acid manages tissue water levels by restricting stomatal opening and activating genes for cell dehydration proteins. The abscisic acid content of inoculated seedlings of *Ormosia hosiei* with *Acrocalymma vagum* was significantly higher than that of non-inoculated seedlings [43]. Nevertheless, our results showed higher ABA production in E- genotypes compared to E+ genotypes under drought stress. The present findings confirm the results of Khan et al. [50], that reported in response to drought stress, endogenous ABA content in cucumber plants with endophytic fungi (*Exophiala*) was lower than E- plants. It seems that higher ABA in uninfected genotypes under drought stress is correlated with inhibition of growth and biomass. In contrast, E+ genotypes neutralized detrimental effects of drought by significantly increasing growth attributes compared to uninfected genotypes.

Aquaporin, an essential membrane protein, expertly transports water and regulates water movement and

cellular water balance in plants [76]. On the other hand, when plants are exposed to abiotic stress, aquaporin responds quickly by regulating water transport and by strengthening the antioxidant system in plants, thus reducing H_2O_2 accumulation and membrane damage [77].

Strikingly, our results showed that the *Lptip1;1* and *Lptip1;2* gene expression in E- genotypes were up-regulated under drought conditions. While in the infected genotypes, we found both up-regulation and down-regulation responses of genes expression. In response to soil water scarcity, plants regulate the expression of aquaporins to minimize water flow and preserve tissue turgor. A number of investigators have previously reported the upregulation of aquaporin in response to water stress in different plant species [78–80]. Up-regulation and down-regulation of aquaporin genes in plant-endophyte interactions could improve drought tolerance of a few plants, such as *O. sativa* [61] and *Z. mays* [81]. Our results also support the view that increased aquaporin levels can be related to adaptation to water stress. The wild-type endophyte strain secretes bioactive alkaloids into host plant tissues when the plant is subjected to stress like water scarcity, thereby bolstering the plant's resilience against such stresses. Plants infected with endophytes may thus have greater persistence in a given environment. Understanding the mechanisms of drought resistance in different grasses infected with endophytes provides the possibility of better use of these cultivars in urban green spaces and pastures in arid and semi-arid areas. In our research, screening drought-tolerant plants such as the S10 genotype that require little water could be a promising choice to prevail productivity limitations in areas prone to stress.

Conclusion

Under drought stress, E+ plants have a more heightened response to the stress due to significant alterations in their physiological characteristics. Although alterations in the physiological characteristics under control conditions were similar in all *Lolium* genotypes, significant differences were observed between the screened genotypes under drought stress conditions. The findings of this study indicated that the E+ genotypes were more tolerant to drought stress conditions compared to the E- genotypes, and the morphological characteristics indicating sensitivity to drought stress in these genotypes was observed later than the E- genotypes. In fact, the results showed that in the studied genotypes of *Lolium perenne*, endophyte infection is advantageous under drought stress but not under non-stress conditions. However, one of the strategies applied by both, uninfected and infected genotypes, in response to the stress, was the accumulation of osmolytes, such as proline. Considering that the E+ genotypes developed more expanded roots than the E- genotypes, they can absorb water more easily from the deeper parts of the soil. The E- genotypes suffered drought stress more than the E+ genotypes and it seems that they responded to this stress by up-regulating aquaporin genes, higher ABA concentration and higher antioxidant capacity (enzymatic and non-enzymatic). The collection of *Lolium perenne* used in this experiment was done from a natural habitat of the plant, and some of the genotypes, especially S10, were superior even over commercial genotypes under drought stress conditions and, therefore, are recommended for further evaluation and possibly utilization as a source of new cultivars development in perennial ryegrass.

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Author contributions
Conceived and designed the experiments: Leila Shabani, Mohammad R. Sabzalian. Performed the experiments: Fatemeh Raeisi Vanani. Analyzed the data: Majid Sharifi-Tehrani. Wrote the paper: Leila Shabani. Edited the manuscript: Leila Shabani, Mohammad R. Sabzalian.

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Data availability
All data generated and analyzed during this study are included in this paper. The raw datasets generated during the current study are also available from the corresponding author on a reasonable request.

Declarations

Ethics approval and consent to participate
Except two commercially available genotypes of Vigor and Speedy which were purchased from the market, seeds of other genotypes of perennial ryegrass were provided by Torkian et al. [24] from plants cultivated in an experimental field in Isfahan University of Technology with full consent and permission of the access. Plants were previously identified by Dr. Ehtemam, the botanist in Isfahan University of Technology, using Flora Iranica. The voucher specimens were deposited in the herbarium of Isfahan University of Technology under the numbers 15 to 22. They are available for botanical studies upon official request.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Falloon P, Betts R. Climate impacts on European agriculture and water management in the context of adaptation and mitigation—the importance of an integrated approach. Sci Total Environ. 2010;408:5667–87. https://doi.org/10.1016/j.scitotenv.2009.05.002
2. White JF, Morgan-Jones G, Morrow AC. Taxonomy, life cycle, reproduction and detection of *Acremonium* endophytes. Agric Ecosyst Environ. 1993;44:13–37.

3. Saikkonen K, Young CA, Helander M, Schardl CL. Endophytic *Epichloë* species and their grass hosts: from evolution to applications. Plant Mol Biol. 2016;90(6). 665– 75. https://doi.org/10.1007/s11103-015-0399-6
4. Redman RS, Freeman S, Clifton DR, Morrel J, Brown G, Rodriguez RJ. Biochemical analysis of plant protection afforded by a non-pathogenic endophytic mutant of *Colletotrichum magna*. Plant Physiol. 1999;119:795–804.
5. Dastogeer KMG, Wylie SJ. Plant–Fungi association: role of fungal endophytes in improving plant tolerance to water stress. In: Singh DP, et al. editors. Plant-Microbe interactions in Agro-Ecological perspectives. Springer Nature Singapore Pte Ltd.; 2017.
6. Tufail MA, Touceda-González M, Pertot I, Ehlers RU. Gluconacetobacter diazotrophicus Pal5 enhances plant robustness status under the combination of moderate drought and low nitrogen stress in *Zea mays* L. Microorganisms. 2021;9:870.
7. Salvi P, Mahawar H, Agarrwal R, Gautam V, Deshmukh R. Advancement in the molecular perspective of plant-endophytic interaction to mitigate drought stress in plants. Front Microbiol. 2022;13:981355. https://doi.org/10.3389/fmicb.2022.981355
8. Dinakar C, Bartels D. Light response, oxidative stress management and nucleic acid stability in closely related Linderniaceae species differing in desiccation tolerance. Planta. 2012;236:541–55. https://doi.org/10.1007/S00425-012-1628-8
9. Dastogeer KMG, Chakraborty A, Alam Sarker MS, Akter MA. Roles of fungal endophytes and viruses in mediating drought stress tolerance in plants. Int J Agric Sci. 2020;24:1497–512.
10. Niu X, Song L, Xiao Y, Ge W. Drought-tolerant plant growth promoting rhizobacteria associated with Foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. Front Microbiol. 2018;8:2580. https://doi.org/10.3389/fmicb.2017.02580
11. Furet PM, Berthier A, Decau ML, Morvan-Bertrand A, Prud'homme MP, Noiraud-Romy N, et al. Differential regulation of two sucrose transporters by defoliation and light conditions in perennial ryegrass. Plant Physiol Biochem. 2012;61:88–96.
12. Turgeon A. Turfgrass management. Copyright by pearson education. New: Inc. Uppere Saddle River; 2002.
13. Kramer P. Water relations of plants. Academic Pres. Inc New York; 1983.
14. Loka D, Harper J, Humphreys M, Gasior D, Wootton-Beard P, Gwynn-Jones D, et al. Impacts of abiotic stresses on the physiology and metabolism of cool-season grasses: A review. Food Energy Secur. 2019;8:e00152. https://doi.org/10.1002/fes3.152
15. Hahn H, McManus MT, Warnsstorff K, Monahan BJ, Young CA, Davies E, Tapper BA, Scott B. *Neotyphodium* fungal endophytes confer physiological protection to perennial ryegrass (*Lolium perenne* L.) subjected to a water deficit. Environ Exp Bot. 2008;63:183–99.
16. Kane KH. Effects of endophyte infection on drought stress tolerance of *Lolium perenne* accessions from the mediterranean region. Environ Exp Bot. 2011;71:337–44.
17. He L, Hatier JHB, Card SD, Matthew C. Endophyte infection reduces leaf dehydration of ryegrass and tall fescue plants under moderate water deficit. Proc N Z Grassland Assn. 2013;75:151–6.
18. Assuero SG, Tognetti JA, Colabelli MR, Agnusdei MG, Petroni EC, Posse MA. Endophyte infection accelerates morpho-physiological responses to water deficit in tall fescue. N. Z. J. Agric. Res. 2006;49:359–70.
19. Crush JR, Popay AJ, Waller J. Effect of different *Neotyphodium* endophytes on root distribution of a perennial ryegrass (*Lolium perenne* L.) cultivar. N. Z. J. Agric. Res. 2004;47:345–9.
20. Hall SL, McCulley RL, Barney RJ, Phillips TD. Does fungal endophyte infection improve tall fescue's growth response to fire and water limitation. PLoS ONE. 2014;9:e86904.
21. Tian Z, Huang B, Belanger FC. Effects of *Epichloe festucae* fungal endophyte infection on drought and heat stress responses of strong creeping red fescue. J Am Soc Hortic Sci. 2015;140:257–64.
22. White RH, Engelke MC, Morton SJ, Johnson-Cicalese JM, Ruemmele BA. *Acremonium* endophyte effects on tall fescue drought tolerance. Crop Sci. 1992;32:1392–6.
23. Dastogeer KMG. Influence of fungal endophytes on plant physiology is more pronounced under stress than well-watered conditions: a meta-analysis. Planta. 2018;248:1403–16.
24. Torkian M, Sabzalian MR, Ehtemam MH. A simultaneous effect of selfing and *Epichloe¨* endophyte on forage, seed yield and turf characteristics of perennial ryegrass (*Lolium perenne* L). Grass Forage Sci. 2019;74:1–12.
25. Saha DC, Jackson MA, Johnson-Cicalese JM. A rapid staining method for detection of endophytic fungi in turf and forage grasses. Phytopathology. 1988;78:237–9.
26. Barrs H, Weatherley P. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Med Sci. 1962;15:413–28.
27. Troll W, Lindsley J. A photometric method for the determination of proline. J Biol Chem. 1955;215:655–60.
28. Alexieva V, Sergiev I, Mapelli S, Karanov E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ. 2001;24:1337–44.
29. Hodges DM, DeLong JM, Forney CF, Prange RK. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta. 1999;207:604–11.
30. Vanani FR, Shabani L, Sabzalian MR, Dehghanian F, Winner L. Comparative physiological and proteomic analysis indicates lower shock response to drought stress conditions in a self-pollinating perennial ryegrass. PLoS ONE. 2020;15:e0234317. https://doi.org/10.1371/journal.pone.0234317.
31. May MJ, Leaver CJ. Oxidative stimulation of glutathione synthesis in *Arabidopsis thaliana* suspension culture. Plant Physiol. 1993;103:157–66.
32. Porter H, Villar R. The fate of acquired carbon in plants: chemical composition and construction costs. In: Fakhri A, Bazzaz JG, editors. Plant resource allocation. San Diego, CA, USA: Academic Press; 1997. p. 39–72.
33. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J Enol Vitic. 1965;16:144–58.
34. Kelen M, Demiralay EC, Sen S, Özkan G. Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99 R (*Vitis berlandieri* x *Vitis rupestris*) and Rose oil (*Rosa damascena*) by reversed phase liquid chromatography. Turk J Chem. 2004;28:603–10.
35. Salehi M, Shabani L, Sabzalian MR. Epichloë endophyte modifies antioxidative defense and aquaporin genes expression in response to ni contamination in lolium perenne. Plant Pro Fun 2021;10(43):55–63.
36. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using Real-Time Quantitative PCR and the $^{2-\Delta\Delta CT}$ Method, Methods. 2001; 25: 402–408.
37. Tang D, Chen M, Huang X, Zhang G, Zeng L, Zhang G, Wu S, Wang Y. SRplot: A free online platform for data visualization and graphing. PLoS ONE. 2023;18:e0294236.
38. De Battista JP, Bouton JH, Bacon CW, Siegel MR. Rhizome and herbage production of endophyte removed tall fescue clones and populations. J Agron. 1990;82:651–4.
39. Han R, Li X, Ren A, Gao Y. Physiological ecological effect of endophyte infection on *Achnatherum sibiricum* under drought stress. Acta Ecol Sin. 2011;31:2115–23.
40. Qiang X, Ding J, Lin W, Li Q, Xu C, Zheng Q, Li Y. Alleviation of the detrimental effect of water deficit on wheat (*Triticum aestivum* L.) growth by an Indole acetic acid-producing endophytic fungus. Plant Soil. 2019;439:373–91.
41. Ren A, Clay K. Impact of a horizontally transmitted endophyte, *Balansia henningsiana*, on growth and drought tolerance of *Panicum rigidulum*. Int J Plant Sci. 2009;170:599–608.
42. Malinowski D, Leuchtmann A, Schmidt D, Nosberger J. Symbiosis with *Neotyphodium uncinatum* endophyte May increase the competitive ability of meadow fescue. Agron J. 1997;89:833–9.
43. Liu Y, Wei X. Dark septate endophyte improves drought tolerance of *Ormosia hosiei* Hemsley & E. H. Wilson by modulating root morphology, ultrastructure, and the ratio of root hormones. Forests. 2019;10:830. https://doi.org/10.3390/f10100830
44. Ferus P, Barta M, Konôpková J. Endophytic fungus *Beauveria bassiana* can enhance drought tolerance in red oak seedlings. Trees. 2019;33:1179–86.
45. Li X, He C, He X, Su F, Hou L, Ren Y, Hou Y. Dark septate endophytes improve the growth of host and non-host plants under drought stress through altered root development. Plant Soil. 2019;439:259–72.
46. Kuzhuppillymyal-Prabhakarankutty L, Tamez-Guerra P, Gomez-Flores R, Rodriguez-Padilla MC. Ek-Ramos, endophytic *Beauveria bassiana* promotes drought tolerance and early flowering in corn. World J Microbiol Biotechnol. 2020;36:1–10.
47. Malinowski DP, Belesky DP. *Epichloë* (formerly *Neotyphodium*) fungal endophytes increase adaptation of cool-season perennial grasses to environmental stresses, Acta Agrobot. 2019; 72: 1767.
48. Man D, Bao YX, Han LB, Zhang XZ. Drought tolerance associated with proline and hormone metabolism in two tall fescue cultivars. HortScience. 2011;46:1027–32.

49. Nagabhyru P, Dinkins RD, Wood CL, Bacon CW, Schardl CL. Tall fescue endophyte effects on tolerance to water-deficit stress. BMC Plant Biol. 2013;13:127.
50. Khan AL, Hamayun M, Ahmad N, Waqas M, Kang SM, Kim YH, et al. *Exophiala sp*. LHL08 reprograms *Cucumis sativus* to higher growth under abiotic stresses. Physiol Plant. 2011;143:329–43. https://doi.org/10.1111/j.1399-3054.2011.01508.x.
51. Molina-Montenegro MA, Oses R, Torres-Diaz C, Atala C, Zurita-Silva A. S. Ruiz-Lara. Root-endophytes improve the ecophysiological performance and production of an agricultural species under drought condition. AoB Plants. 2016; 8:plw062.
52. Ren AZ, Gao YB, Wang W, Wang JL. Photosynthetic pigments and photosynthetic products of endophyte-infection and endophyte-free *Lolium perenne* L. under drought stress conditions. Front Biol Chin. 2006;25:225–31.
53. Yang T, Ma S, Dai CC. Drought degree constrains the beneficial effects of a fungal endophyte on *Atractylodes lancea*. J Appl Microbiol. 2014;117:1435–49.
54. Dastogeer KMG, Li H, Sivasithamparam K, Jones MGK, Wylie SJ. A simple and rapid in vitro test for large-scale screening of fungal endophytes from drought-adapted Australian wild plants for conferring water deprivation tolerance and growth promotion in *Nicotiana benthamiana* seedlings. Arch Microbiol. 2017;199:1357–70.
55. Afshari M, Rahimmalek M, Sabzalian MR, Miroliaei M, Szumny A. Essential oil profiles, improvement of enzymatic and non-enzymatic antioxidant systems in different populations of *Salvia subg. Perovskia* by irrigation management. Ind Crops Prod. 2024;208:117849. https://doi.org/10.1016/j.indcrop.2023.117849.
56. Azad K, Kaminskyj S. A fungal endophyte strategy for mitigating the effect of salt and drought stress on plant growth. Symbiosis. 2016;68:73–8.
57. Xu L, Li X, Han L, Li D, Song G. *Epichloe* endophyte infection improved drought and heat tolerance of tall fescue through altered antioxidant enzyme activity. Eur J Hortic Sci. 2017;82:90–7.
58. Zhang YP, Nan ZB. Germination and seedling anti-oxidative enzymes of endophyte-infected populations of *Elymus dahuricus* under osmotic stress. Seed Sci Technol. 2010;38:522–7.
59. Khan AL, Waqas M, Hamayun M, Al-Harrasi A, Al-Rawahi A, Lee IJ. Co-synergism of endophyte *Penicillium resedanum* LK6 with salicylic acid helped *Capsicum annuum* in biomass recovery and osmotic stress mitigation. BMC Microbiol. 2013;13:1–13.
60. Guler NS, Pehlivan N, Karaoglu SA, Guzel S, Bozdeveci A. *Trichoderma atroviride* ID20G inoculation ameliorates drought stress-induced damages by improving antioxidant defence in maize seedlings. Acta Physiol Plant. 2016;38:132–40.
61. Pandey V, Ansari MW, Tula S, Yadav S, Sahoo RK, Shukla N, et al. Dose-dependent response of *Trichoderma harzianum* in improving drought tolerance in rice genotypes. Planta. 2016;243:1251–64.
62. Cheynier V, Comte G, Davies KM, Lattanzio V, Martens S. Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiol Biochem. 2013;72:1–20. https://doi.org/10.1016/j.plaphy.2013.05.009.
63. Pratyusha S. Phenolic compounds in the plant development and defense: An overview. In Plant Stress Physiology—Perspectives in Agriculture; Hasanuzzaman, M., Nahar, K., Eds.; IntechOpen: London, UK, 2022.
64. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot. 2003;91:179–94.
65. Arora A, Byrem TM, Nair MG, Strasburg GM. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Arch Biochem Biophys. 2000;373:102–9.
66. Karuppanapandian T, Moon JC, Kim C, Manoharan K, Kim W. Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. AJCS. 2011;5:709–25.
67. Sharma P, Dubey RS. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regul. 2005;46:209–21.
68. Chakraborty U, Chakraborty BN, Chakraborty AP, Dey PL. Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria. World J Microbiol Biotechnol. 2013;29:789–803.
69. Khan G. Enhancement and drought tolerance of hybrid Poplar upon inoculation with endophyte consortia. Curr. Plant Biol. 2016;6:38–47.
70. Moghaddam MSH, Safaie N, Soltani J, Hagh-Doust N. Desert-adapted fungal endophytes induce salinity and drought stress resistance in model crops. Plant Physiol Biochem. 2021;160:225–38. https://doi.org/10.1016/j.plaphy.2021.01.022.
71. Ghaffari MR, Mirzaei M, Ghabooli M, Khatabi B, Wu Y, Zabet-Moghaddam M, et al. Root endophytic fungus *Piriformospora indica* improves drought stress adaptation in barley by metabolic and proteomic reprogramming. Environ Exp Bot. 2019;157:197–210.
72. Siddiqui MH, Al-Khaishany MY, Al-Qutami MA, Al-Whaibi MH, Grover A, Ali HM, Bukhari NA. Response of different genotypes of faba bean plant to drought stress. Int J Mol Sci. 2015;16:10214–27.
73. Dong C, Fu Y, Liu G, Liu H. Growth, photosynthetic characteristics, antioxidant capacity and biomass yield and quality of wheat (*Triticum aestivum* L.) exposed to LED light sources with different spectra combinations. J Agron Crop Sci. 2014;200:219–30.
74. Hasanuzzaman M, Nahar K, Fujita M, Ahmad P, Chandna R, Prasad MNV, et al. Enhancing plant productivity under salt stress—Relevance of Poly-Omics. In: Ahmad P, Azooz MM, Prasad MNV, editors. Salt stress in plants: omics, signaling and responses. Berlin, Germany: Springer; 2013. pp. 113–56.
75. Dar NA, Amin I, Wani W, Wani SA, Shikari AB, Wani SH, Masoodi KZ. Abscisic acid: A key regulator of abiotic stress tolerance in plants. Plant Gene. 2017;11:106–11.
76. Yi X, Sun X, Tian R, Li K, Ni M, Ying J, Xu L, Liu L, Wang Y. Genome-wide characterization of the aquaporin gene family in radish and functional analysis of RsPIP2-6 involved in salt stress. Front Plant Sci. 2022;13:860742. https://doi.org/10.3389/fpls.2022.860742
77. Hu W, Yuan Q, Wang Y, Cai R, Deng X, Wang J, et al. Overexpression of a wheat aquaporin gene, TaAQP8, enhances salt stress tolerance in transgenic tobacco. Plant Cell Physiol. 2012;53:2127–41. https://doi.org/10.1093/pcp/pcs154
78. Alexandersson E, Fraysse L, Sjovall-Larsen S, Gustavsson S, Fellert M, Karlsson M, et al. Whole gene family expression and drought stress regulation of aquaporins. Plant Mol Biol. 2005;59:469–84.
79. Aroca R, Ferrante A, Vernieri P, Chrispeels MJ. Drought, abscisic acid and transpiration rate effects on the regulation of PIP aquaporin gene expression and abundance in *Phaseolus vulgaris* plants. Ann Bot Lond. 2006;98:1301–10.
80. Yue C, Cao H, Wang L, Zhou Y, Hao X, Zeng J, et al. Molecular cloning and expression analysis of tea plant aquaporin (AQP) gene family. Plant Physiol. Biochem. 2014;83:65–76.
81. Gong M, Bai N, Wang P, Su J, Chang Q, Zhang Q. Co-inoculation with arbuscular mycorrhizal Fungi and dark septate endophytes under drought stress: synergistic or competitive effects on maize growth, photosynthesis, root hydraulic properties and aquaporins. Plants. 2023;12:2596. https://doi.org/10.3390/plants12142596

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