Eur. J. Hortic. Sci. *82*(2), 90–97 | ISSN 1611-4426 print, 1611-4434 online | https://doi.org/10.17660/eJHS.2017/82.2.4 | © ISHS 2017

Original article

Epichloe endophyte infection improved drought and heat tolerance of tall fescue through altered antioxidant enzyme activity

Lixin Xu[1], Xiaoshuai Li[1], Liebao Han[1], Deying Li[2] and Guilong Song[1]

[1] Institute of Turfgrass Science, Beijing Forestry University, Beijing 100083, China
[2] North Dakota State University, Fargo 58102, North Dakota, United States

Summary

Background of the study: Epichloe **species are fungal endophytes of many cool season grass species. The *Epichloe*-tall fescue association is generally known to confer drought tolerance of tall fescue although the effect varied depending a lot of factors. Besides, few studies are focused on the effect of fungal endophyte on heat tolerance of tall fescue. *Objectives:* This study was to investigate the effect of fungal endophyte on drought and heat tolerance of tall fescue. *Methods:* In this study, endophyte infected (E+) and non-infected (E-) tall fescue plants were exposed to two separate experiments, drought tolerance experiment and heat tolerance experiment. Physiological parameters and antioxidant enzyme activity were measured. *Results:* Compared with E- plants, lower levels of electrolyte leakage, malondialdehyde content, and hydrogen peroxide (H_2O_2) content were observed in E+ plants during drought, re-watering and heat stress treatment. Lower level of superoxide (O_2-) content was also observed in E+ plants upon re-watering. In addition, endophyte infection promoted higher relative water content and higher superoxide dismutase (SOD) and ascorbate peroxidase (APX) activity at the end of drought treatment. E+ plants had higher levels of catalase (CAT), guaiacol peroxidase (POD) and APX activity than E- plants by the end of heat treatment. *Conclusions:* Our results support a protective role of the endophyte in conferring drought and heat tolerance of tall fescue. E+ plants appear to reduce ROS (reactive oxygen species, e.g., O_2- and H_2O_2) production in part by maintaining higher enzyme activity of SOD and APX under drought and CAT, POD and APX activity under heat stressed conditions.**

Keywords
ascorbate peroxidase, catalase, *E. typhina*, hydrogen peroxide, malondialdehyde, reactive oxygen species, relative water content, superoxide

Abbreviations
RWC, relative water content; EL, electrolyte leakage; SOD, superoxide dismutase; CAT, catalase; POD, guaiacol peroxidase; APX, ascorbate peroxidase; MDA, malondialdehyde; ROS, reactive oxygen species

Significance of this study

What is already known on this subject?

- The fungal endophyte-tall fescue association is generally considered to be mutualistic and the infection improved plants abiotic and biotic stress tolerance with few reports showing not the case.

What are the new findings?

- *E. typhina* improved drought and heat tolerance of tall fescue. The mechanism of better drought and heat tolerance of *E. typhina* endophyte infected tall fescue plants is likely related to less ROS mediated damage and promotion of specific antioxidant enzyme activity in the host plant by the endophyte.

What is the expected impact on horticulture?

- Our results indicated that utilizing endophyte is a possible way of improving drought and heat tolerance of tall fescue. In addition, the paper will aid understanding the mechanism of endophyte mediated drought and heat tolerance of tall fescue.

Introduction

Epichloe species are fungal endophytes of many cool season grass species (such as *Festuca* spp. and *Lolium* spp.) (Schardl et al., 1994; Tsai et al., 1994). The species are growing asymptomatically within the plant tissues for nutrition. Grasses infected with *Epichloe* species can gain profound fitness enhancements due, in part, to fungal alkaloids providing biological protection from herbivores and insects to the grass (Czarnoleski et al., 2012; Schardl, 1996). *Epichloe* endophytes have also been found to have a beneficial role in host plants responses to abiotic stress conditions, such as salinity (Yin et al., 2014), Ni stress (Mirzahossini et al., 2015), and Cd stress (Ren et al., 2011).

Drought and heat are the most widespread abiotic stress encountered by plants. Enhanced drought tolerance by *Epichloe coenophialum* infection in tall fescue (*Festuca arundinacea*) is supported in many studies (Buck et al., 1997; Elmi and West, 1995; Nagabhyru et al., 2013). Hahn et al. (2008) reported that *Epichloe lolii* improved drought tolerance of perennial ryegrass (*Lolium perenne*). However, there are other reports showing no evidence for endophyte-mediated drought tolerance (Hall et al., 2014; Tian et al., 2015; White et al., 1992). Therefore, the endophyte effects on drought responses in tall fescue were not consistent across all tall fescue genotypes (Assuero et al., 2000). This complexity reflects

the interaction between host genotype, endophyte strain and water status variables (Assuero et al., 2006). There are little studies on the effect of fungal endophyte infection on the responses of the host plants to heat stress. Tian et al. (2015) revealed that *E. fetucae* infection had no physiological effect of endophyte on improving heat tolerance of strong creeping red fescue plants.

While the biology and various effects of fungal endophytes on host plants were widely studied, the mechanisms responsible for the effect are not well understood. To date, endophyte enhanced drought tolerance is demonstrated to be associated with altered water potential, enhanced osmotic adjustment (free sugars, sugar alcohols, amino acids), survival and growth of infected compared to non-infected plants (Buck et al., 1997; Elbersen and West, 1996; Elmi and West, 1995; Hahn et al., 2008). Some other reports revealed that endophyte ameliorated the effects of drought stress through promoting higher antioxidant enzyme activity and accumulating metabolites of phenolics, carbohydrates, mannitol, and proline with antioxidant capacity which protect plants from oxidative stress under water-deficit conditions (Gundel et al., 2012; Hamilton et al., 2012; Malinowski and Belesky, 2000; Rodriguez et al., 2008, 2009).

Tall fescue (*Festuca arundinacea*) is an important forage and turfgrass species which is widely used in Beijing and northern China for lawn and athletic fields. Naturally, *E. coenophiala*, formerly being designated as *Neotyphodium. coenophialum* (Leuchtmann et al., 2014), is a systemic and seed-transmissible endophyte of tall fescue (Arachevaleta et al., 1989). Overall, the effect of this endophyte has been confirmed to be positive in improving plants' stress tolerance. In this experiment, in order to test if the effect of another endophyte strain *E. typhina* (*N. typhinum*) (Leuchtmann et al., 2014) is the same on tall fescue, a commercial cultivar 'Millennium' was artificially infected by *E. typhina*. Drought and heat treatment were imposed separately to study the effect of *E. typhina* on tall fescue. Physiological parameters such as relative water content (RWC), and electrolyte leakage (EL) were examined to determine if the presence of the endophyte does confer to the host grass any significant degree of tolerance to drought and heat. Content of superoxide (O_2-), hydrogen peroxide (H_2O_2) and MDA and antioxidant enzyme activity were further investigated to determine key factors involved in endophyte enhanced drought and heat tolerance for tall fescue.

Materials and methods

Seeds of the tall fescue cultivar 'Millennium' were obtained from the seeds company Top Green (Beijing, China). Endophyte-infected (designated as E+) plants were generated by inoculating the endophyte-free cultivar 'Millennium' with *E. typhina* (isolated from *Festuca ovina*) (Wang et al., 2009). Both endophyte-free (designated as E-) and endophyte-infected E+ plants of tall fescue were then grown on Turfgrass Research Farm of Beijing Forestry University (40°10'34"N, 116°14'52"E). Soil pH was 8.05. Sand to clay ratio was 3.5:1. After 2-year growth, plants were transferred to plastic pots (Sand to clay ratio was 3.5:1) in a greenhouse. Day/night temperatures were 23/16°C and average photoperiod was 14-h light at 400 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR). Relative humidity was 40%. Plants were trimmed weekly to 12–14 cm and watered regularly. The endophyte status of the plants was monitored during vegetative propagation and immediately before the stress experiment by staining leaf sheath with rose bengal as described by Saha et al. (1988). Tissues from leaf sheath of plants were sampled for investigation. A leaf sheath from the plant was removed and the adaxial epidermis was cut aseptically with a sterile scalpel and then stripped with sterile forceps. The epidermal tissue was placed on a microscope slide and dyed with a drop of 1 g L^{-1} cotton blue in lacto-phenol. The endophytic mycelium was observed by microscopic examination with 250–400 of magnification.

For drought tolerance experiment, after 5-week acclimation to greenhouse conditions, plants were exposed to 3 treatments: 1) well-watered control treatment: plants were watered every 2 d to maintain soil volumetric water content (SWC) at 40.4%; 2) drought-stress treatment: irrigation was withheld for 15 d and SWC dropped gradually to 4% at the end of drought treatment and 3) re-watering treatment: after drought, plants were re-watered daily for a total of 10 d to maintain SWC at about 40%. Each treatment had 5 replicates (5 pots of plants). All pots were kept well-watered until water stress initiation. SWC was measured as an indication of the level of soil water deficit. Soil moisture in the 0–6 cm soil layer of each pot was measured using the soil-moisture probe Theta Probe type ML2x and meter type HH2 (Delta-T Devices, Cambridge, UK).

For heat tolerance experiment, plants were transferred from greenhouse to growth chambers. Average photoperiod was 14-h light at 400 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR). Relative humidity was 50%. Day/night temperatures were 23/16°C. Plants were watered every 2 days to maintain soil water content at 38%. After 4 weeks acclimation in growth chamber, plants were exposed to 2 growth chambers: for control treatment: Day/night temperatures were 23/16°C and for heat stress treatment: Day/night temperatures were 38/32°C for 35 days. Each treatment had 4 replicates (4 pots of plants).

Physiological changes were evaluated by measuring RWC and cellular membrane stability expressed as EL. Leaf RWC was determined based on three weights of collected tissue; fresh weight (FW) (about 0.15 g), dry weight (DW) and turgid weight (TW) using the formula RWC (%) = [(FW−DW)/(TW−DW)]×100. Leaves were collected from plants and immediately weighed for determination of FW. Samples were then immersed in de-ionized water and kept in dark for 12 h at 4°C after which they were removed from deionized water, gently blotted dry, and weighed for TW. Samples were then dried in an oven set to 80°C for at least 72 h and again weighed for DW determination (Barrs and Weatherley, 1962). To measure EL, fresh leaves (0.1 g) were collected, rinsed with deionized water to remove external solutes, and immersed in 20 mL de-ionized water. Initial conductivity of the solution ($C_{initial}$) was measured using a conductivity meter (www.sh-leici.com, Corporation of China, Shanghai, China) following incubation in de-ionized water for 24 h on a shaker. Leaf samples were then killed by autoclaving at 121°C for 15 min and cooled at room temperature for final conductivity (C_{max}) measurement. EL percentage was then calculated as ($C_{initial}/C_{max}$)×100 (Blum and Ebercon, 1981).

The formation rate of superoxide radicals (O_2-) was determined at 530 nm according to the method of Elstner and Heupel (1976) and was calculated from a standard curve of KNO_2. For hydrogen peroxide (H_2O_2) content determination, 0.25 g of fresh leaves were homogenized in 5 mL of refrigerated acetone and were centrifuged at 3,000 *g* for 20 min, the supernatant was used for the H_2O_2 content assay as described by Patterson et al. (1984). 4 mL of cold-acetone-extracted supernatant was added to 0.5 mL 20% titanium re-

agents (20% (w/v) $TiCl_4$ in 12.1 M HCl and 1 mL 17 M ammonia solution. The solution was centrifuged at 10,000 g_n at 4°C for 5 minutes, and the supernatant was discarded. The pellet was dissolved in 4 mL of 1 M sulfuric acid. Absorbance of the solution was measured at 410 nm with a spectrophotometer. Absorbance values were calibrated to a standard curve generated with known concentrations of H_2O_2, which were expressed in µmol g^{-1} FW.

Determination of enzyme activity was carried out using the method previously described by Xu et al. (2011a). Briefly, 0.25 g of fresh leaf tissue was ground to a fine powder using a mortar and pestle and extraction was performed with 4 mL of extraction buffer (50 mM potassiumphosphate, 1 mM- ethylenediaminetetraacetic acid, 1% polyvinylpyrrolidone, and 1 mM dithiothreitol, pH of 7). The extractions were centrifuged at 15,000 g_n for 30 min at 4°C, and supernatant was collected for enzyme assay and malondialdehyde (MDA) content measurement. The superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by recording the rate of p-nitro blue tetrazoliuE- chloride reduction in absorbance at 560 nm (Giannopolitis and Ries, 1977). The activity of catalase (CAT, EC 1.11.7.6), guaiacol peroxidase (POD, EC 1.11.1.7), and ascorbate peroxidase (APX, EC 1.11.1.1) was determined by following the changes in absorbance at 240, 470, and 290 nm respectively. Absorbance change of 0.1 in 1 min was defined as one unit of enzyme activity.

The lipid peroxidation was measured in terms of MDA content (Dhindsa and Matowe, 1981) with some modifications. A 1-mL aliquot of supernatant was mixed with 0.67% thiobarbituric acid. The mixture was heated at 95°C for 15 min, quickly cooled on ice, and then centrifuged at 10,000 *g* for 5 min. The absorbance of the supernatant was read at 532 and 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Hatier et al., 2014).

Effects of stress treatment, grass infection status (E- or E+) and corresponding interactions were determined by analysis of variance according to the general linear model procedure of SAS (Version 9.0; SAS Institute, Cary, NC). Differences between means were separated by Fisher's protected least significance difference test at the 0.05 P level.

Results

Physiological changes

Leaf RWC showed no difference between E- and E+ plants under well-watered conditions and upon re-watering (Figure 1A). RWC decreased drastically at 15 d of drought treatment while it decreased more in E- plants (22.6% in E- plants compared to 32.9% in E+ plants). Drought treatment increased EL levels for both E- and E+ plants. E- plants increased more, from about 5.7% to 89%. E+ plants increased from 5.4% to 73% by the end of drought treatment (Figure 1B). Compared to E- plants, E+ plants recovered better in terms of EL upon re-watering. Similarly, heat stress also increased EL value. E+ plants had relative lower value of EL being 36% compared to E- plants being 46% at the end of heat treatment (Figure 1C).

ROS accumulation and lipid peroxidation

Measurement of O_2^- formation rate and H_2O_2 content indicated significant increases under drought stress and subsequent decreases upon re-watering (Figures 2A and B). Notably, E- plants had more O_2^- upon re-watering compared to E+ plants. H_2O_2 content was higher in E- plants under drought and re-watering conditions. Drought causes significant lipid peroxidation (Figure 2C) (shown as increased MDA content) in E- and E+ plants. Re-watering treatment resulted in restoration in terms of MDA content for both types of plants (Figure 2C) while E+ plants had significantly lower MDA content than E- plants. The presence of endophyte reduced O_2^- under re-watering, and it decreased H_2O_2 and MDA content during drought and re-watering. Heat stress increased O_2^- formation rate (Figure 2D), H_2O_2 (Figure 2E) and MDA content (Figure 2F) in both plants. The presence of endophyte reduced O_2^-, H_2O_2 and MDA content under heat stressed conditions.

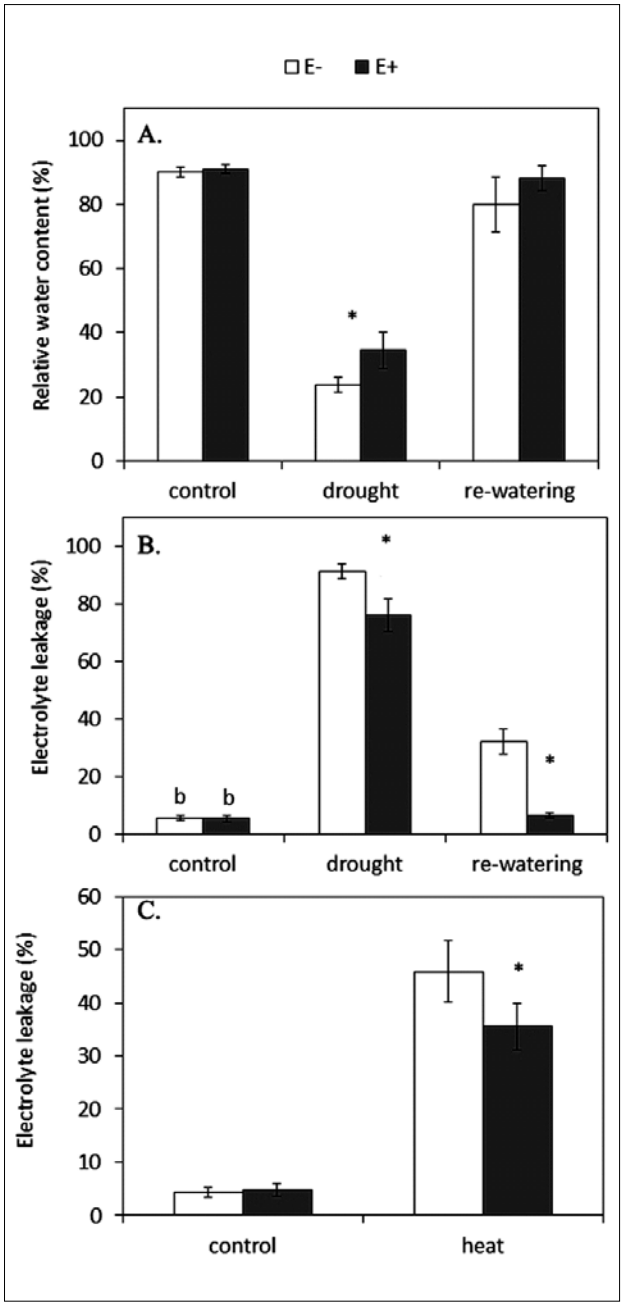


Figure 1. Physiological changes in E- and E+ plants. Physiological responses of endophyte infected (E+) and non-infected (E-) plants in drought tolerance (Figure 1A, B) and heat tolerance experiment (Figure 1C). Bars indicate ±SE ($n = 5$ in drought tolerance experiment; $n = 4$ in heat tolerance experiment). Asterisks indicate significant difference existed between E+ and E- plants ($P \leq 0.05$).

Antioxidant enzyme activity

Under drought stressed conditions, the presence of endophyte increased SOD (Figure 3A) and APX (Figure 3B) activity in tall fescue. Under well-watered conditions, endophyte infection increased APX (Figure 3B) and CAT (Figure 3C) activity in tall fescue while it did not affect SOD and CAT activity. In heat stressed conditions, the presence of endophyte increased CAT (Figure 4A), POD (Figure 4B), and APX (Figure 4C) activity while SOD (Figure 4D) activity was not affected.

Discussion

Physiological changes

RWC is an important parameter for drought resistance selection in winter wheat and turf grasses (Almeida et al., 2014; Schonfeld et al., 1988; Xu et al., 2013). Endophyte infection promoted higher RWC in plants indicating that *E. typhina* infection played a beneficial role to tall fescue plants in maintaining higher level of leaf RWC in response to drought. Zhang and Nan (2007) found that endophyte infected *Elymus dahuricus* plants maintained higher RWC of plants under

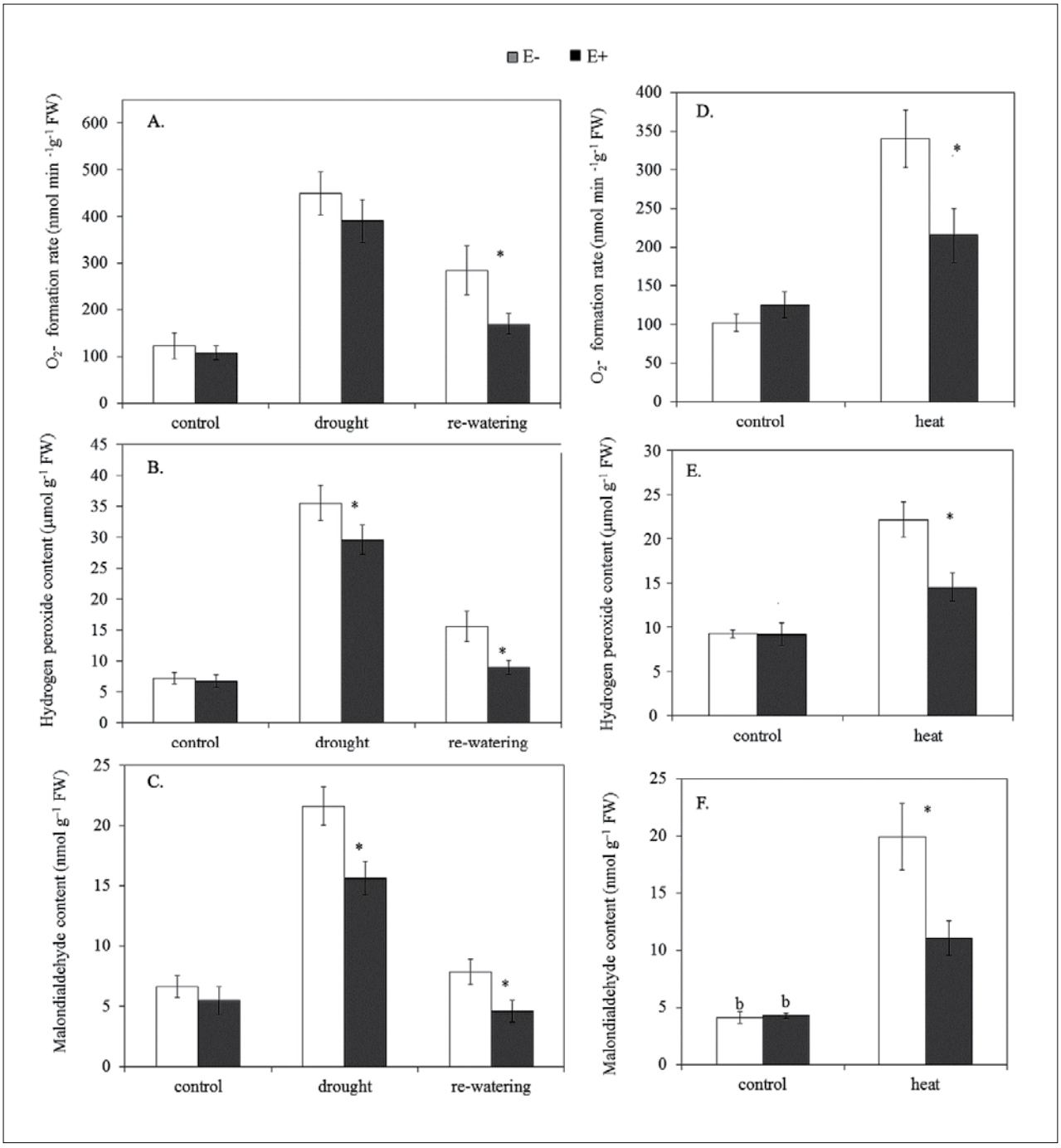


Figure 2. ROS accumulation and lipid peroxidation in E- and E+ plants. Reactive oxygen species (O_2- and hydrogen peroxide) and lipid peroxidation (malondialdehyde) changes in endophyte infected (E+) and non-infected (E-) plants in drought (Figure 2A – C) and heat tolerance experiment (Figure 2D – F). Bars indicate ± SE (n = 5 in drought tolerance experiment; n = 4 in heat tolerance experiment). Asterisks indicate significant difference existed between E+ and E- plants ($P \leq 0.05$).

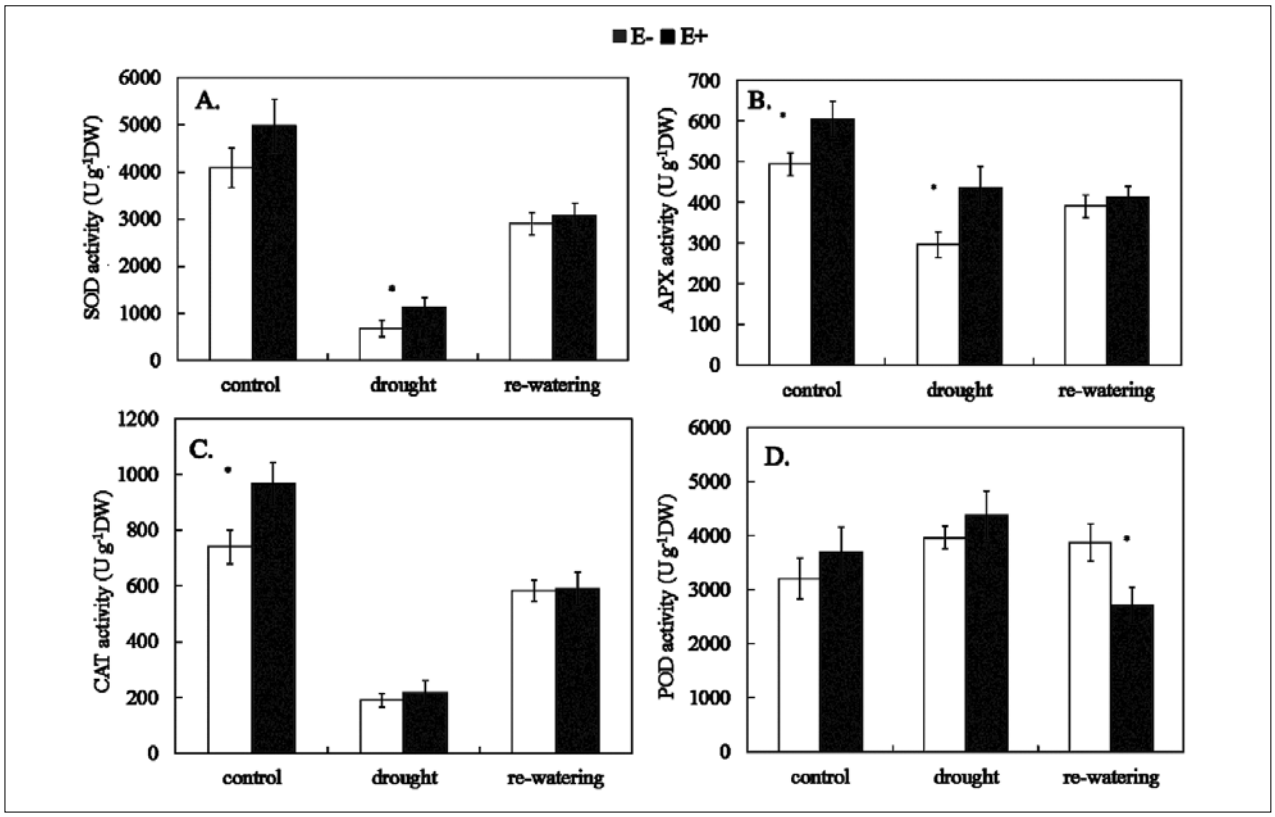


FIGURE 3. Antioxidant enzyme activity changes in drought tolerance experiment. Superoxide dismutase (SOD) (Figure 3A), ascorbate peroxidase (APX) (Figure 3B), catalase (CAT) (Figure 3C) and guaiacol peroxidase (POD) (Figure 3D) changes in endophyte infected (E+) and non-infected (E-) plants in drought tolerance experiment. Bars indicate ± SE ($n = 5$). Asterisks indicate significant difference existed between E+ and E- plants ($P \leq 0.05$).

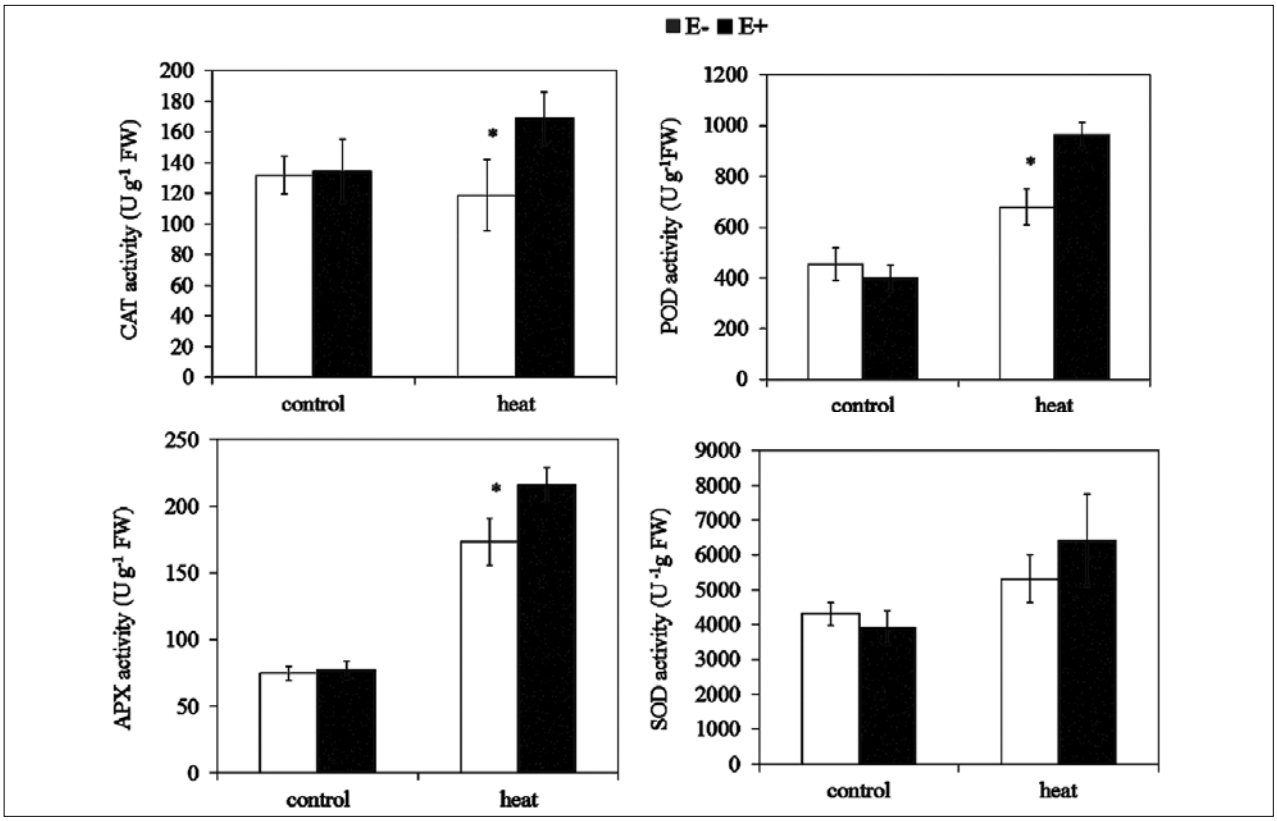


FIGURE 4. Antioxidant enzyme activity changes in heat tolerance experiment. Catalase (CAT) (Figure 4A), guaiacol peroxidase (POD) (Figure 4B), ascorbate peroxidase (APX) (Figure 4C), and superoxide dismutase (SOD) (Figure 4D) changes in endophyte infected (E+) and non-infected (E-) plants in heat tolerance experiment. Bars indicate ± SE ($n = 4$). Asterisks indicate significant difference existed between E+ and E- plants ($P \leq 0.05$).

mild water deficit conditions, which was in support with our results. Inconsistent with our results, natural *E. coenophiala* infected tall fescue (Bayat et al., 2009) and synthetic *E. coenophiala* tall fescue symbiotum (Assuero et al., 2000; White et al., 1992) were reported with no difference observed in RWC compared with endophyte-free plants under water-stressed conditions. In different studies, plants were exposed to different intensity and duration of water stress which might be reasons causing contrasting results. Endophyte might work in a complex way in affecting leaf water relations under limited water conditions. The intensity and duration of drought, endophyte species and other factors together play important roles in this process.

Leaf EL is used as an indicator of cell membrane stability during abiotic and biotic stress. Cell membrane stability is critical for maintaining cellular functions under abiotic stress conditions (Blum and Ebercon, 1981; Marcum, 1998; Xu et al., 2011b). For perennial plant species, rapid and complete post-drought recovery is one of the most important strategies after drought when water is again available and high recovery ability may be an important and economical selecting trait in turfgrass breeding programs (de Aldana et al., 2014). Endophyte improved tall fescue cell membrane stability during drought, re-watering and heat treatment. The underlying mechanism might be that endophyte presence provides better antioxidant capacity which protects cell membrane from ROS damage (Hamilton et al., 2012).

ROS accumulation and lipid peroxidation

Over-production of ROS (hydrogen peroxide, superoxide radicals, hydroxyl radicals and singlet oxygen) are induced by various abiotic stresses in plants. ROS are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress (Yeh and Kirschner, 2014). As the result of the endophyte-plant symbiosis, E+ plants presented lower content of H_2O_2 during drought, re-watering and heat treatment indicating better H_2O_2 scavenging ability in E+ plants. In addition, E+ plants had lower O_2^- formation rate during re-watering suggesting a possible supportive role of endophyte in scavenging O_2^- which might be critical for plants recovery from severe drought stress. Lower levels of MDA content were observed in E+ plants exposed to drought, re-watering and heat treatment indicating less lipid peroxidation resulted from ROS damage. Our results again suggested better ROS protection in E+ plants than in E- plants under both drought- and heat-stressed conditions.

Antioxidant enzyme activity

Plants have evolved various enzymatic mechanisms to detoxify harmful ROS, including SOD, CAT, POD, and APX (Marquez et al., 2007; Rosa et al., 2009; Sugawara et al., 2009). SOD is the first line of defense against oxidative stress in plants and is responsible for scavenging superoxide to produce hydrogen peroxide, reducing the risk of highly reactive hydroxyl radical formation from superoxide through the metal-catalyzed Haber-Weiss-type reaction (Smirnoff, 1993). APX is involved in Ascorbate-Glutathione (AsA-GSH) cycle which is an alternative and more effective detoxification mechanism against H_2O_2 operating both in chloroplasts and the cytosol (Marquez et al., 2007; Rosa et al., 2009). APX requires a reductant-ascorbate substrate and has a higher affinity for H_2O_2, therefore allowing efficient scavenging of H_2O_2 in more specific locations (Marquez et al., 2007). The higher SOD activity and APX activity observed in E+ plants under drought condition suggested that endophyte presence promoted higher ROS scavenging capacity during drought stress. Although the mechanisms remain unknown, higher activities of CAT, POD and APX were observed in E+ plants under heat treatment. Increases in various antioxidants have been repeatedly shown to correlate with increased plant tolerance to multiple stresses (Hamilton et al., 2012). Our results indicated that endophyte infection improved heat tolerance of tall fescue in part by promoting higher CAT, POD and APX activity.

Conclusions

Results suggest that artificially fungal endophyte infection can help host tall fescue plants to alleviate abiotic stress such as drought and heat. The mechanism of better drought and heat tolerance of endophyte infected tall fescue plants is likely related to less ROS mediated damage. Thus, promotion of specific antioxidant enzyme activity in the host plant by the endophyte, for example, SOD and APX by drought, CAT, POD and APX by heat seems to be important in protecting from ROS damages and providing resistance to the grass.

Acknowledgments

This work was supported by the Fundamental Research Funds for the Central Universities and grant 2013AA102607 from the MOST of China 863 Fund. We wish to thank Yaoyao Wang and Dr. Yongji Du for technical assistance. Thanks also go to Dr. David Jespersen, Dr. Patrick Burgess and anonymous reviewers for critical reading of the manuscripts.

References

Almeida, C., Ortega, H., Higginbotham, S., Spadafora, C., Arnold, A.E., Coley, P.D., et al. (2014). Chemical and bioactive natural products from *Microthyriaceae* sp., an endophytic fungus from a tropical grass. Lett. Appl. Microbiol. *59*, 58–64. https://doi.org/10.1111/lam.12245.

Arachevaleta, M., Bacon, C.W., Hoveland, C.S., and Radcliffe, D.E. (1989). Effect of the tall fescue endophyte on plant response to environmental stress. Agron. J. *81*, 83–90. https://doi.org/10.2134/agronj1989.00021962008100010015x.

Assuero, S.G., Matthew, C., Kemp, P.D., Latch, G.C.M., Barker, D.J., and Haslett, S.J. (2000). Morphological and physiological effects of water deficit and endophyte infection on contrasting tall fescue cultivars. New Zeal. J. Agr. Res. *43*, 49–61. https://doi.org/10.1080/00288233.2000.9513408.

Assuero, S.G., Tognetti, J.A., Colabelli, M.R., Agnusdei, M.G., Petroni, E.C., and Posse, M.A. (2006). Endophyte infection accelerates morpho-physiological responses to water deficit in tall fescue. New Zeal. J. Agr. Res. *49*, 359–370. https://doi.org/10.1080/00288233.2006.9513726.

Barrs, H., and Weatherley, P. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. *15*, 413–428. https://doi.org/10.1071/BI9620413.

Bayat, F., Mirlohi, A., and Khodambashi, M. (2009). Effects of endophytic fungi on some drought tolerance mechanisms of tall fescue in a hydroponics culture. Russ. J. Plant Physiol. *56*, 510–516. https://doi.org/10.1134/S1021443709040104.

Blum, A., and Ebercon, A. (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. *21*, 43–47. https://doi.org/10.2135/cropsci1981.0011183X002100010013x.

Buck, G.W., West, C.P., and Elbersen, H.W. (1997). Endophyte effect on drought tolerance in diverse *Festuca* species. In Neotyphodium/

Grass Interactions, C. Bacon, and N. Hill, eds. (US: Springer), p. 141–143. https://doi.org/10.1007/978-1-4899-0271-9_21.

Czarnoleski, M., Pawlik, K., Olejniczak, P., Kozowski, J., and Lembicz, M. (2012). An endophytic fungus reduces herbivory in its recently colonised grass host: a food-choice experiment on common voles, weeping alkaligrass and *Epichloe typhina*. Plant Ecol. *213*, 1049–1053. https://doi.org/10.1007/s11258-012-0064-y.

de Aldana, B.R.V., Gundel, P.E., Criado, B.G., Ciudad, A.G., Sanchez, A.G., and Zabalgogeazcoa, I. (2014). Germination response of endophytic *Festuca rubra* seeds in the presence of arsenic. Grass Forage Sci. *69*, 462–469. https://doi.org/10.1111/gfs.12049.

Dhindsa, R.S., and Matowe, W. (1981). Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. J. Exp. Bot. *32*, 79–91. https://doi.org/10.1093/jxb/32.1.79.

Elbersen, H.W., and West, C.P. (1996). Growth and water relations of field-grown tall fescue as influenced by drought and endophyte. Grass Forage Sci. *51*, 333–342. https://doi.org/10.1111/j.1365-2494.1996.tb02068.x.

Elmi, A.A., and West, C.P. (1995). Endophyte infection effects on stomatal conductance, osmotic adjustment and drought recovery of tall fescue. New Phytol. *131*, 61–67. https://doi.org/10.1111/j.1469-8137.1995.tb03055.x.

Elstner, E.F., and Heupel, A. (1976). Inhibition of nitrite formation from hydroxylammoniumchloride: A simple assay for superoxide dismutase. Anal. Biochem. *70*, 616–620. https://doi.org/10.1016/0003-2697(76)90488-7.

Giannopolitis, C.N., and Ries, S.K. (1977). Superoxide dismutases: I. Occurrence in higher plants. Plant Physiol. *59*, 309–314. https://doi.org/10.1104/pp.59.2.309.

Gundel, P.E., Hamilton, C.E., Seal, C.E., Helander, M., Alejandra Martinez-Ghersa, M., Ghersa, C.M., et al. (2012). Antioxidants in *Festuca rubra* L. seeds affected by the fungal symbiont *Epichloe festucae*. Symbiosis *58*, 73–80. https://doi.org/10.1007/s13199-012-0194-y.

Hahn, H., McManus, M.T., Warnstorff, K., Monahan, B.J., Young, C.A., Davies, E., et al. (2008). Neotyphodium fungal endophytes confer physiological protection to perennial ryegrass (*Lolium perenne* L.) subjected to a water deficit. Environ. Exp. Bot. *63*, 183–199. https://doi.org/10.1016/j.envexpbot.2007.10.021.

Hall, S.L., McCulley, R.L., Barney, R.J., and Phillips, T.D. (2014). Does fungal endophyte infection improve tall fescue's growth response to fire and water limitation? PLoS One *9*, e86904. https://doi.org/10.1371/journal.pone.0086904.

Hamilton, C.E., Gundel, P.E., Helander, M., and Saikkonen, K. (2012). Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. Fungal Diversity *54*, 1–10. https://doi.org/10.1007/s13225-012-0158-9.

Hatier, J.H.B., Faville, M.J., Hickey, M.J., Koolaard, J.P., Schmidt, J., Carey, B.L., et al. (2014). Plant vigour at establishment and following defoliation are both associated with responses to drought in perennial ryegrass (*Lolium perenne* L.). J. Exp. Bot. *65*, 5823–5834. https://doi.org/10.1093/jxb/eru318.

Leuchtmann, A., Bacon, C.W., Schardl, C.L., White, J.F., and Tadych, M. (2014). Nomenclatural realignment of *Neotyphodium* species with genus *Epichloë*. Mycologia *106*, 202–215. https://doi.org/10.3852/13-251.

Malinowski, D.P., and Belesky, D.P. (2000). Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Sci. *40*, 923–940. https://doi.org/10.2135/cropsci2000.404923x.

Marcum, K.B. (1998). Cell membrane thermostability and whole-plant heat tolerance of Kentucky Bluegrass. Crop Sci. *38*, 1214–1218. https://doi.org/10.2135/cropsci1998.0011183X003800050017x.

Marquez, S.S., Bills, G.F., and Zabalgogeazcoa, I. (2007). The endophytic mycobiota of the grass *Dactylis glomerata*. Fungal Diversity *27*, 171–195.

Mirzahossini, Z., Shabani, L., Sabzalian, M.R., and Sharifi-Tehrani, M. (2015). ABC transporter and metallothionein expression affected by NI and *Epichloe* endophyte infection in tall fescue. Ecotox. Environ. Safe. *120*, 13–19. https://doi.org/10.1016/j.ecoenv.2015.05.025.

Nagabhyru, P., Dinkins, R.D., Wood, C.L., Bacon, C.W., and Schardl, C.L. (2013). Tall fescue endophyte effects on tolerance to water-deficit stress. BMC Plant Biology *13*, 127. https://doi.org/10.1186/1471-2229-13-127.

Patterson, B.D., MacRae, E.A., and Ferguson, I.B. (1984). Estimation of hydrogen peroxide in plant extracts using titanium (IV). Anal. Biochem. *139*, 487–492. https://doi.org/10.1016/0003-2697(84)90039-3.

Ren, A.Z., Li, C.A., and Gao, Y.B. (2011). Endophytic fungus improves growth and metal uptake of *Lolium arundinaceum* Darbyshire Ex. Schreb. Int. J. Phytoremediat. *13*, 233–243. https://doi.org/10.1080/15226511003671387.

Rodriguez, R.J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., et al. (2008). Stress tolerance in plants via habitat-adapted symbiosis. Isme Journal *2*, 404–416. https://doi.org/10.1038/ismej.2007.106.

Rodriguez, R.J., White, J.F., Jr., Arnold, A.E., and Redman, R.S. (2009). Fungal endophytes: diversity and functional roles. New Phytol. *182*, 314–330. https://doi.org/10.1111/j.1469-8137.2009.02773.x.

Rosa, L.H., Vaz, A.B.M., Caligiorne, R.B., Campolina, S., and Rosa, C.A. (2009). Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv. (Poaceae). Polar Biol. *32*, 161–167. https://doi.org/10.1007/s00300-008-0515-z.

Saha, D.C. (1988). A rapid staining method for detection of endophytic fungi in turf and forage grasses. Phytopathology *78*, 237–239. https://doi.org/10.1094/Phyto-78-237.

Schardl, C.L. (1996). *Epichloe* species: fungal symbionts of grasses. Annu. Rev. Phytopathol. *34*, 109–130. https://doi.org/10.1146/annurev.phyto.34.1.109.

Schardl, C.L., Leuchtmann, A., Tsai, H.F., Collett, M.A., Watt, D.M., and Scott, D.B. (1994). Origin of a fungal symbiont of perennial ryegrass by interspecific hybridization of a mutualist with the ryegrass choke pathogen, *Epichloe typhina*. Genetics *136*, 1307–1317.

Schonfeld, M.A., Johnson, R.C., Carver, B.F., and Mornhinweg, D.W. (1988). Water relations in winter wheat as drought resistance indicators. Crop Sci. *28*, 526–531. https://doi.org/10.2135/cropsci1988.0011183X002800030021x.

Smirnoff, N. (1993). The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. *125*, 27–58. https://doi.org/10.1111/j.1469-8137.1993.tb03863.x.

Sugawara, K., Hesse, U., Liu, J., Goodin, M.M., Barnes, A., Dinkins, R.D., et al. (2009). The grass symbiont, *Epichloe festucae*, visualized in endophytic and pathogenic states by GFP expression with epifluorescence and confocal microscopy. Phytopathology *99*, S126–S126.

Tian, Z., Huang, B., and Belanger, F.C. (2015). Effects of *Epichloe festucae* fungal endophyte infection on drought and heat stress responses of strong creeping Red Fescue. J. Am. Soc. Hortic. Sci. *140*, 257–264.

Tsai, H.F., Liu, J.S., Staben, C., Christensen, M.J., Latch, G.C., Siegel, M.R., et al. (1994). Evolutionary diversification of fungal endophytes of tall fescue grass by hybridization with *Epichloe* species. P. Natl. Acad. Sci. U.S.A. *91*, 2542–2546. https://doi.org/10.1073/pnas.91.7.2542.

Wang, Y., Du, Y., Han, L., and Li, D. (2009). Endophytes from natural *Festuca* spp. in southwest China and their compatibility with tall fescue cultivars. Kor. Turfgrass Sci. *23*(1), 163–176.

White, R.H., Engelke, M.C., Morton, S.J., Johnson-Cicalese, J.M., and Ruemmele, B.A. (1992). Acremonium Endophyte effects on tall fescue drought tolerance. Crop Sci. *32*, 1392–1396. https://doi.org/10.2135/cropsci1992.0011183X003200060017x.

Xu, L., Han, L., and Huang, B. (2011a). Antioxidant enzyme activities and gene expression patterns in leaves of Kentucky bluegrass in response to drought and post-drought recovery. J. Am. Soc. Hortic. Sci. *136*, 247–255.

Xu, L., Han, L., and Huang, B. (2011b). Membrane fatty acid composition and saturation levels associated with leaf dehydration tolerance and post-drought rehydration in Kentucky bluegrass. Crop Science *51*, 273–281. https://doi.org/10.2135/cropsci2010.06.0368.

Xu, L., Yu, J., Han, L., and Huang, B. (2013). Photosynthetic enzyme activities and gene expression associated with drought tolerance and post-drought recovery in Kentucky bluegrass. Environ. Exp. Bot. *89*, 28–35. https://doi.org/10.1016/j.envexpbot.2012.12.001.

Yeh, Y.H., and Kirschner, R. (2014). *Sarocladium spinificis*, a new endophytic species from the coastal grass *Spinifex littoreus* in Taiwan. Bot. Stud. *55*, 25. https://doi.org/10.1186/1999-3110-55-25.

Yin, L., Ren, A., Wei, M., Wu, L., Zhou, Y., Li, X., et al. (2014). *Neotyphodium coenophialum*-infected tall fescue and its potential application in the phytoremediation of saline soils. Int. J. Phytoremediat. *16*, 235–246. https://doi.org/10.1080/15226514.2013.773275.

Zhang, Y.P., and Nan, Z.B. (2007). Growth and anti-oxidative systems changes in *Elymus dahuricus* is affected by *Neotyphodium* endophyte under contrasting water availability. J. Agron. Crop. Sci. *193*, 377–386. https://doi.org/10.1111/j.1439-037X.2007.00279.x.

Received: Jun. 21, 2016
Accepted: Jan. 31, 2017

Addresses of authors:
Lixin Xu[1], Xiaoshuai Li[1], Liebao Han[1], Deying Li[2] and Guilong Song[1,*]
[1] Institute of Turfgrass Science, Beijing Forestry University, Beijing 100083, China
[2] North Dakota State University, 1241 N. University Dr., Fargo 58102, North Dakota, United States
* Corresponding author; E-mail: syihan@163.com
Box 116, 35 Tsinghua East Rd, Beijing 100083, China
Tel.: 8610-62336408; Fax: 8610-62336399