Fungal Ecology 30 (2017) 39–47

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Effect of an *Epichloë* endophyte on adaptability to water stress in *Festuca sinensis*

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ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form
3 August 2017
Accepted 28 August 2017

Corresponding Editor: Nicole Hynson

Keywords:
Epichloë endophytes
Festuca sinensis
Plant performance
Soil water content
Drought
Waterlogging

ABSTRACT

As water stress, including drought and waterlogging, can severely affect plant growth, this study investigated the effects of an endophyte from the genus *Epichloë* on two different ecotypes of *Festuca sinensis* grass under five soil water conditions in a controlled greenhouse experiment. Changes in *F. sinensis* plants grown with (E+) and without the endophyte (E−) were evaluated as they were subjected to different water treatments (20%, 35%, 50%, 65% and 80% relative saturation moisture content, RSMC). Growth parameters such as plant height, number of tillers, blade width, stem diameter, root length, total biomass, root-shoot ratio and relative water content were determined. The results showed that drought and waterlogging significantly ($P < 0.05$) inhibited the growth of *F. sinensis*. The presence of the endophyte significantly ($P < 0.05$) increased plant growth and root-shoot ratio under drought and waterlogged conditions. In addition, the plant height, number of tillers, blade width, stem diameter and total biomass in seedlings of both ecotypes reached the maximum at 65% RSMC, which suggests the optimal water condition. These findings also show that moderate drought (35% and 50% RSMC) could promote root growth of grass seedlings. Therefore, endophytic infections can result in enhanced host plant resistance to drought and waterlogged conditions.

1. Introduction

The establishment and survival of plants in semi-arid regions mainly depends on soil water conditions. Plants usually grow well under optimal soil moisture content, but their growth and reproduction can be limited due to unfavourable (high or low) osmotic potential (Neil and Faeth, 2003). Given the globally changing climate, drought problems are expected to be a serious concern in the near future (Bacon, 1993; Balba, 1995; Bates et al., 2008). In many arid and semi-arid regions, water deficit has become one of the most persistent and damaging problems for plant growth, causing ion imbalances, and affecting the transportation and accumulation of plant nutrients, resulting in nutrient and metabolic disorders of plants (Kuldau and Bacon, 2008; Rong et al., 2015; Song et al., 2015a,b). Therefore, it is necessary to understand how plants respond to drought or waterlogging conditions and explore strategies to maintain the production and functions of the ecosystem, particularly under the context of climate change.

Endophytic fungi belonging to the genera *Epichloë* have been found in many cool-season grasses, especially in forage and turf grasses and wild grasses of meadows (Chen et al., 2015; Leuchtmann et al., 2014; Zhang et al., 2015a,b). The mutualistic symbiotic relationship between endophytic fungi and grasses has been extensively studied. *Epichloë* species can systemically infect their host plants and inhabit the above-ground tissues of grasses while remaining asymptomatic (Stone et al., 1996; Neil and Faeth, 2003; Schardl et al., 2004; Li et al., 2006). Endophytes obtain their nutrients from the shoot meristem of their hosts and grow synchronously with their host plants (Christensen et al., 2008; Kuldau and Bacon, 2008). Additional research has shown that host plants provide a spatial structure, food security and transmission of nutrients to the endophyte (Malinowski and Belesky, 2000; Clay and Schardl, 2002; Nan and Li, 2004; Xia et al., 2016). The endophyte, in return, provides benefits to the host plant by enhancing plant growth, increasing resistance to a wide range of abiotic and biotic stresses and improving in-field persistence (Clay and Schardl, 2002; Li et al., 2006; Nagabhyru et al., 2013; Ma et al.,

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http://dx.doi.org/10.1016/j.funeco.2017.08.005
1754-5048/

2014; Matsukura et al., 2014; Oberhofer et al., 2014; Song et al., 2015a,b; Wakelin et al., 2015; Wang et al., 2015; Xia et al., 2016). However, endophytes can also produce secondary metabolites like alkaloids that have detrimental effects on animal health (Clay and Schardl, 2002; Nan and Li, 2004). Therefore, studying the endophyte in detail has become very important.

Festuca sinensis is an important wild forage grazed by cattle and sheep in alpine pastoral areas, especially in the cool and semi-arid regions of China (Zhou et al., 2015a,b). It is also an important cool-season grass species involved in grassland establishment and ecological management programs in the Qinghai-Tibetean Plateau of China (Li et al., 2010; Tian et al., 2015). This grass species is frequently infected by an asexual asymptomatic *Epichloë sp.*, and accompanied by *Elymus* species (Zhou et al., 2015a,b). Recent studies have reported that *Epichloë* can not only improve seed germination and seedling growth in *F. sinensis* but also enhance the ability of the host grasses to resist cold stress (Peng et al., 2012; Zhou et al., 2015a,b). However, there are no reports of endophytic associations in *F. sinensis* that cause toxicity in grazing animals. This suggests that it has great potential for non-toxic endophyte breeding programs. Very little is known about the endophytic effects on *F. sinensis* and how it influences stress response of host plants. To gain a comprehensive understanding of the endophytic effects on *F. sinensis*, the present study examines the performance of two different ecotypes: *F. sinensis* with (E+) and without endophyte (E−) under different soil moisture conditions to evaluate the effects of both endophyte and water stress on host grasses, and to gain insights into the mechanisms through which endophytes affect water stress tolerance in *F. sinensis*.

2. Materials and methods

2.1. Plant materials

The seeds of *F. sinensis* were obtained from the Institute of Grassland, Qinghai Academy of Animal Husbandry and Veterinary Sciences, which were originally collected from the natural grasslands in two different townships of Ping'an County, Qinghai Province, China (36°20.021′ N, 102°06.241′ E, Altitude 3129 m (09–41) and 36°20.508′ N, 102°06.348′ E, Altitude 2994 m (09–57), respectively) during the summer of 2013 and dispatched to Lanzhou University in January of 2014. The well-filled, healthy-looking seeds were planted and the endophyte in seedlings were detected by microscopic examination of host leaf sheath stained with aniline blue (Nan, 1996). The labelled plants (E+ and E−) were transported to the experimental field at Yuzhong Pastoral Agriculture Experimental Station of Lanzhou University (35°56′ N, 104°08′ E, Altitude 1718 m) in March 2014. Harvesting was performed on 25th August 2015, and *F. sinensis* seeds were harvested from E+ and E− plants and stored at a constant 5 °C for the present study. On the 29th August, 2015, healthy and well-filled seeds from one E+ plant and one E− plant were planted into a 48-hole plastic seedling tray containing sterilized vermiculite and grown until emergence.

2.2. Experimental design

Four weeks after sowing, the seedlings having similar growth were transplanted from the seedling trays to pots containing the same amount of media (sterilized sand and natural soil in a w/w ratio 1:2) and the natural soil collected from an experimental field of Lanzhou University. Each pot (diameter: 15 cm; height: 21 cm) had only one seedling and equal initial water treatment. The infection status of each seedling was also determined by aniline blue staining (Nan, 1996) and the plants were distinguished by marking as E+ and E−. The endophytic fungal infection rates of the two ecotypes were 95%(E+), 0%(E−) (09–41) and 97%(E+), 0%(E−) (09–57), respectively. The E+ and E− seedlings that had been evaluated were chosen for further studies. All the seedlings were equally irrigated (50 ml per pot) daily to maintain the same soil moisture content until October 30, 2015.

Pot-based experiments in a controlled-environment were performed from 30th October 2015 to 30th January 2016 (3 months) in the greenhouse at the College of Pastoral Agriculture Science and Technology, Lanzhou University. On 30th October, 2015, five different water-holding capacities were established, which included severe drought (20% relative saturation moisture content-RSMC), moderate drought (35% RSMC), light drought (50% RSMC), normal moisture (65% RSMC), and abundant moisture (80% RSMC). A total of 20 treatments, and six replicates per treatment were used in this experiment. All pots contained uniform-sized seedlings. Plants in each replicate used for different treatments were from the same seed-derived plant. These pots were randomly placed in a greenhouse maintained at a constant temperature (temperature: 22 ± 2 °C, moisture: 42 ± 2%) and 11 h light and 13 h dark cycle. During the experiment, each pot was weighed and watered every evening at 6 p.m. to maintain the appropriate soil moisture content, i.e. at 20%, 35%, 50%, 65% or 80% RSMC. After watering, each pot was randomly placed until the experiment ended.

2.3. Measurement protocols

2.3.1. Plant performance

On January 30, all plants included in this study were carefully removed from the experimental pots, washed with distilled water and dried using a filter paper. The plant height, root length and tiller number of each plant from each treatment were determined, the blade width and stem diameter per plant were measured using Vernier callipers (Mitutoyo, Japan). During this measurement, all harvested plants were processed and separated into roots and foliage components and the fresh weight of shoots and roots were recorded. Dry weight was obtained after oven-drying the tissue at 80 °C until a constant weight was reached. Each of the dry aboveground parts and roots from each treatment were weighed separately to determine total dry matter per plant.

2.3.2. Root-shoot ratio

The root-shoot ratio was calculated by dividing the root dry weight by the combined dry weight of all above-ground plant parts.

2.3.3. Relative water content

The fresh and dry weights were used to determine the relative water content (RWC) of the plants. Fresh weight (FW) was determined after separating the plants into two parts, and then dry weight (DW) was determined after drying the leaves to a constant weight in an oven at 105 °C, followed by leaf turgid-weight (TW) which was determined by rehydrating the leaves in the dark for 24 h (Lafitte, 2002). RWC was calculated using the following formula: $RWC = [(FW - DW)/(TW - DW)] \times 100$.

2.4. Statistical analysis

Data analyses were performed with SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) to analyse the effects of *F. sinensis* ecotype, effects of the endophyte and soil water content on different growth parameters. A three-way ANOVA was performed. A repeated-measures ANOVA with Fisher's least significant differences (LSD) test was used to determine whether differences between means were statistically significant. If the means of various experimental parameters were significantly different between E+ and E− plants, then significance of difference between E+ and E− plants in all