Effects of Epichloë endophyte infection on growth, physiological properties and seed germination of wild barley under saline conditions

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Effects of *Epichloë* endophyte infection on growth, physiological properties and seed germination of wild barley under saline conditions

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Abstract

Background and aims Wild barley (*Hordeum brevisubulatum*) is a grass that inhabits alkalized meadows in northern China. An asexual *Epichloë bromicola* endophyte was detected in seeds and leaf sheaths in all wild barley samples from Gansu Province, China. The aim of this research was to determine the effects of the *Epichloë* endophyte on growth, physiological properties and seed germination of wild barley under salt stress.

Methods In field plots and pot experiments, endophyte-infected (E+) and endophyte-free (E-) plants were subjected to two salt treatments (soil salt contents of 3% and 0.03%) for 16 weeks (field plots) and 8 weeks (pots), after which plant growth and physiological properties were investigated. In a seed germination experiment, using seeds collected from the field plot experiment, E+ and E- seeds were grown in different NaCl treatments (0, 100, 200, 300 and 400 mM) for 2 weeks, after which seed germination parameters were measured.

Results E+ plants produced more tillers, with a higher ratio of reproductive tillers and higher biomass than E- plants under high salt stress. Chlorophyll content and superoxide dismutase (SOD) activity in E+ plants were increased, and malondialdehyde (MDA) content in E+ plants was reduced under high salt stress. Seed germination parameters of E+ plants were significantly higher than those of E- plants when NaCl concentration reached 200 and 300 mM.

Conclusions Our results demonstrate that *Epichloë* endophytes increased tolerance to salt stress in wild barley by increasing seed germination and growth, and altering plant physiology. We suggest that the endophyte could increase yield and salinity resistance in wild barley.

Key words: *Hordeum brevisubulatum*; *Epichloë bromicola*; Biomass; Seed yield; Saline stress

Abbreviations: E+, endophyte-infected; E-, endophyte-free; LS, low salt; HS, high salt; RTR, relative tillering rate; TKW, thousand-kernel weight; ISTA, International Seed Testing Association.

Introduction

Salinity is a worldwide problem in agricultural production and is a major abiotic stress for plants in semi-arid or arid regions; a third of irrigated land and a fifth of cropland are affected by salinity

(Muscolo et al. 2003; Shrivastava and Kumar 2015). Wild barley (*Hordeum brevisubulatum*), a perennial forage species, has strong salinity resistance and good productivity. It is the dominant species in alkalized meadows in northern China (Wang 2003) and has been widely cultivated in Jilin, Inner Mongolia, Hebei, Gansu, Qinhai and Xinjiang provinces of China (Jia 1987). In addition, numerous wild barley lines have been used to provide saline-alkali recovery and utilization. By reason of its wide geographic distribution, economic importance and strong salinity resistance, wild barley is one of the best grasses to study response to saline environments.

Epichloë endophytes are fungi that can infect and co-exist with host plants and have been shown to impart tolerance to certain biological and abiotic stresses in a range of host grasses (Li et al. 2004; Malinowski et al. 2005a; Seto et al. 2007; Redman et al. 2011; Wang et al. 2017). Some fungal endophytes facilitate plant growth even in harsh environments (Rodriguez et al. 2009; Yang et al. 2013). Studies have reported that endophyte presence affects plant growth, increasing tiller number, biomass production and seed yield (Kimmons et al. 1990; Azevedo et al. 1995; Hesse et al. 2003; Vila-Alub et al. 2005). However, not all endophytes show the same benefits in hosts under all conditions. For instance, Faeth et al. (2004) found that endophytes did not affect the seed germination of Arizona fescue [*F. arizonica* Vasey (Pooideae)]; and Eerens et al. (1998) and Cheplick et al. (2000) did not find enhanced growth due to endophytes under conditions of their experiments.

Research on salt tolerance of hosts bearing *Epichloë* endophytes has received significant attention in recent years. It was reported that under salt stress, endophyte infection was beneficial to roots of ryegrass (*Lolium perenne* L.) (Ren et al. 2006), and drunken horse grass (*Achnatherum inebrians*) infected with *Epichloë gansuensis* (*Neotyphodium gansuense*) had faster seedling growth and higher seed germination rate under salt stress (Gou 2007). However, endophytes may also decrease salt tolerance in their hosts. For instance, the endophyte frequency in a population of *Roegnaria* samples from an saline-alkali area decreased as the concentration of saline-alkali in the sampling zone increased in Dongying, Shandong Province, China (Wang et al. 2005). One study indicated that endophytes did not influence biomass production of *F. rubra* under soil salinity conditions (Zabalogeazcoa et al. 2006).

In order to maximize plant production in the breeding of forage species, tiller number per plant, plant height and total dry weight are important factors to consider. To increase seed production in plants important factors include ratio of reproductive tillers to non-reproductive tillers, numbers of inflorescences per plant, length of each inflorescence per plant, seeds per inflorescence and seed yield. To evaluate the hardiness of plants under salt stress, plants may be assessed for chlorophyll content, superoxide dismutase (SOD) activity and malondialdehyde (MDA) content. Germination rate of seeds reflects plant fitness, and germination ability may also have a crucial role to play in establishment growth of plants under saline-alkali stress.

Epichloë endophyte infection of *H. brevisubulatum* plants in 13 populations in China has been reported to range from 67% to 100% (Nan and Li 2000; Wang 2009). Several researchers have investigated the physiological function of *Epichloë* endophyte infection in *H. brevisubulatum* plants under soil salt stress and studies have shown that *Epichloë* endophytes could enhance salt tolerance (Wang 2009; Song et al. 2015; Chen et al. 2018a). However, it is still unknown how the *Epichloë* endophyte in *H. brevisubulatum* plants affects forage yield, seed output, new generation seed germination under saline field conditions. Thus, to expand our understanding of the breeding of *Hordeum brevisubulatum* plants bearing the endophyte, we investigated the endophyte-infected (E+) and endophyte-free (E-) plants for growth, seed production, physiological properties, and seed germination parameters under salt stress.

Materials and methods

Plant material

Hordeum brevisubulatum seeds were collected from wild plants in August 2008 from Linze (Altitude: 1580 m, E: 100°06′, N: 39°11′), Zhangye City, Gansu Province, China. Seeds were assessed for *Epichloë bromicola* endophyte infection through microscopic examination using stain 0.8% aniline blue (Li et al. 2004; Chen et al. 2018b). Of the 100 plants evaluated, 100% were found to be E+. To obtain E- plant material, half of the E+ seeds (from one plant) of *Epichloë* endophyte-infected wild barley were treated using benomyl fungicide for 1 h to produce E- seeds (Cheplick et al. 1989). To reduce impacts of the fungicide treatment, E- and E+ plants were grown in the field for two years. The infection status of all plants was confirmed by microscopic examination (Li et al. 2008) before the beginning of the experiment. After harvest, E+ and E- seeds were stored at 5 °C in the seed storage room of the Lanzhou Official Seed Testing Center, Ministry of Agriculture, China. Plastic pots (180 mm height, 140 mm diameter) were filled with loess (2.5 kg/pot) that had been autoclaved at 120 °C for 25 min. Five seeds were distributed over the surface of the soil and covered with a thin layer of soil. Plastic pots were placed in a greenhouse (15 °C – 25 °C) with 14 h of illumination (800 µmol m^{-2} s^{-1}) per day and watered twice weekly. Four weeks after sowing, the infection status of seedlings was tested by microscopic examination of leaf sheath with aniline blue stain (Li et al. 2004).

Field experiment

Each experimental plot (240 cm in length, 240 cm in width, and 50 cm in height) consisted of loess soil that had no history of growing crops, and were located on the campus of the College of Pastoral Agriculture Science and Technology (Altitude: 1520 m, E: 103°36′, N: 36°28′), Lanzhou University, Lanzhou, China. The experiment began on 13 April 2010 when plants with five tillers were transplanted into field with one of two salt stress: low salt stress [LS: soil salt content of 0.03% (i.e. 0.03 g NaCl per 100 g soil)] and high salt stress [HS: soil salt content of 3% (i.e., additional NaCl was added into soil to increase the salt content of soil to 3% or to make a salt content of 3 g NaCl per 100 g soil), using a Soil-EC Tester, Beijng, China. Each salt stress included two type endophyte treatments (i.e., E+ and E-). The experiment consisted of 4 blocks with 3 plots nested in each, and 2 rows plants within each plot. Both row distance and plant distance is 20 cm. Plants were watered daily for the first week after transplanting to support establishment, then once a week thereafter. Two plants (marked) were randomly sampled from each plot at week 4, 8 or 16, and plant height and number of tillers per plant were measured. The relative tillering rate (RTR) was calculated as $(lnT_2 - lnT_1) / 70d$, where T_1 is the tiller number at week 4 and T_2 is the tiller number at week 16, following Tomas (1980). Plants were harvested on 20 August 2010. The total biomass was determined after over-dried at 60 ℃ for 3 days. The seed yield, number of inflorescences per plant, thousand-kernel weight (TKW) and ratio of reproductive tillers were also measured at the end of experiment, as confirmed according to the International Seed Testing Association (ISTA) rules (ISTA 1999).

Pot experiment

The pot experiment began on 1 September 2017, in a greenhouse (environmental conditions: 15 to 20 °C, 60-70% relative humidity, and 14-hr/10-hr light/dark period, light intensity 800 µmol m^{-2} s^{-1}) of Baiyin Agricultural Science Research Institute (Altitude: 1702 m, E: 105°34′, N: 37°38′), Baiyin, China. Seeds of E+ and E- plants were harvested from the field experiment. Plastic pots were filled with soil treated with salt stress as described in the field experiment. Five E+ and five E- seeds were sown in separate plastic pots. Hence, the pot experiment involved 2 salt stress levels × 2 endophyte types × 6 replicates (pots) × 5 plants per pot, resulting in a total of 120 plants. Eight weeks after the start of experiment, we determined chlorophyll content, malondialdehyde (MDA) concentration and superoxide dismutase (SOD) activities of the E+ and E- plants to estimate the possible endophyte effects on salt stress physiological reactions. Chlorophyll content was tested according to the procedures described by Arnon (1967): 0.1 g of plant leaves were extracted in 80% acetone and centrifuged at 4000 rpm for 20 min; and then the sample of supernatant liquid was measured at 663 and 645 nm wavelengths for Chl a and Chl b, respectively. Superoxide dismutase (SOD) activity was detected in leaf tissue (0.05 g in FW) as described by Giannopolitis and Ries (1977). Malondialdehyde (MDA) content was determined according to the thio-barbituric acid (TBA) reaction method (Puckette et al. 2007) using a spectrophotometer (SP-723; Shanghai, China). Malondialdehyde (MDA) concentration was expressed as millimoles per gram fresh weight (FW).

Seed germination experiment

Seeds of wild barley were collected from E+ and E- plants that grew in the experimental field. After dried in natural sunlight, seeds were stored at 5 °C for 6 months to break dormancy. The effect of NaCl (NaCl concentrations = 0, 100, 200, 300 and 400 mM) on seed germination of wild barley was done in a germination chamber (Fang et al. 2006) with alternating temperatures of 15/25 °C (12 h AM / 12 h PM) and with a 80% relative humidity. These humidity and temperature conditions are optimal for wild barley germination (Yu et al. 1999). In this experiment seeds were germinated on filter paper involving 5 salt concentrations × 2 endophyte types × 6 replicates per concentration × 100 seeds per replicate, resulting in a total of 6000 seeds. Germination of seeds was assessed daily for 14 days. The numbers of germinated seeds were counted, and the lengths of the roots and coleoptiles were measured.

Statistical analysis

All analyses were performed using SPSS version 19.0 software (SPSS Inc., Chicago, IL). Prior to ANOVA, data were checked for normality and homogeneity, and were transformed whenever necessary to meet the assumptions. Statistical significance was defined at the 95% confidence level. The parameters of vegetative, reproductive growth, physiological variables (i.e., chlorophyll, MDA and SOD), seed germination, coleoptile length and root length were analyzed using two-way ANOVA, with salt concentrations, endophyte treatment, and their interactions as fixed factors (Table 1). Means among treatments were compared based on the Tukey test ($p < 0.05$) (Figures 1-4).

Results

Endophyte enhances growth under salt stress

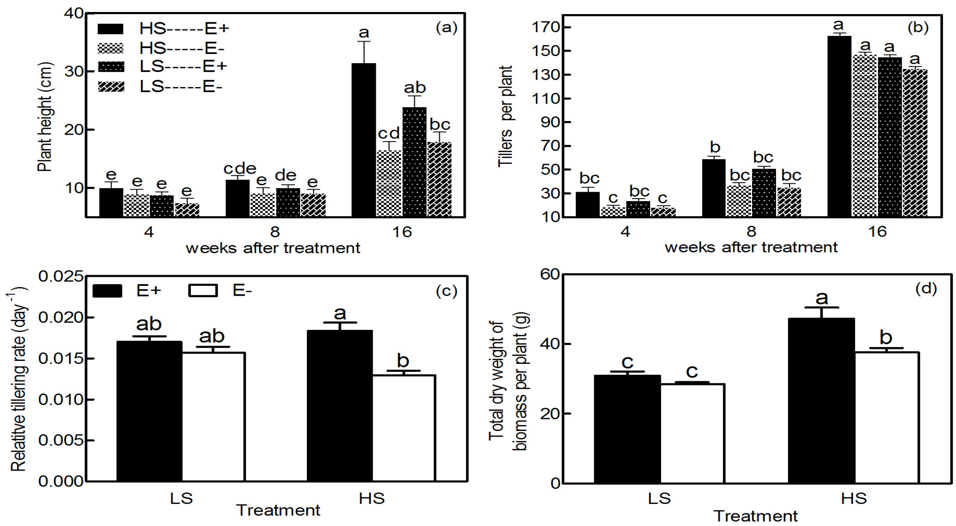

Fig.1 Plant height (a), tiller number per plant (b), relative tillering rate (c), and total dry weight of biomass per plant (d) of *H. brevisubulatum* per plant as affected by endophyte infection (E+ and E-) and high (HS) or low (LS) salt content (values are Means ± SE). Different letters indicate significant at $p < 0.05$ among treatments (Tukey test).

The plant height and tiller number of E+ plants were greater than that of E- plants under two salt stress levels at all growth stages (Fig.1a, b). Endophyte infection caused a significant increase in plant height, and E+ plants were significantly greater than that of E- plants at week 16 under HS stress (Fig.1b and Table 1). E+ plants had approximately 41% higher relative tillering rate (RTR) and 25% more biomass than E- plants under HS stress; no remarkable difference in RTR and plant biomass was detected between E+ and E- plants in the LS stress (Fig. 1c, d). RTR and total dry weight of biomass per plant were significantly affected by endophyte infection (Table 1). For total dry weight of biomass, the endophyte × salt interaction was also significant (Table 1). These results indicate that the effect of *Epichloë* endophyte on plant growth was affected by salt stress level.

Endophyte enhance yield under salt stress

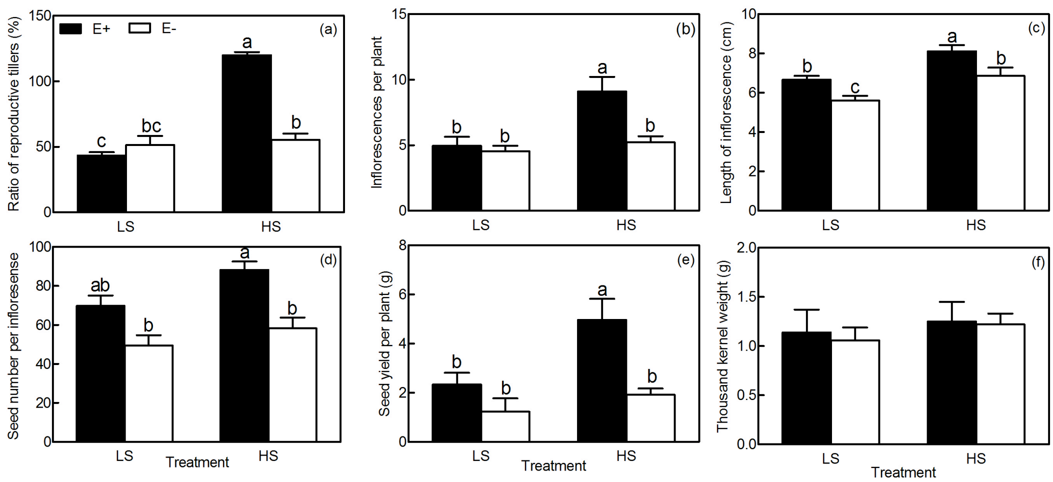

Fig. 2 Ratio of reproductive tillers (a), inflorescences per plant (b), length of inflorescence (c), seed number per inflorescence (d), seed yields per plant (e) and thousand kernel weight (f) of *H. brevisubulatum* per plant as

affected by endophyte infection (E+ and E-) and high (HS) or low salt (LS) content (Values are Means ±SE). Different letters indicate significant at $p < 0.05$ among treatments (Tukey test).

Endophyte infection significantly increased the ratio of reproductive tillers to non-reproductive tillers by 116% in the HS stressed treatment, but the E- plant ratio of reproductive tillers to non-reproductive tillers was more than that of E+ plants under LS treatment, and there was no significant effect between them (Fig. 2a). The results indicated that E+ plants had 43% more inflorescences per plant than E- plants in the HS stress level (Fig. 2b); inflorescences were significantly longer on E+ plants than on E- plants in the case of HS stress and LS stress (18% and 17%, respectively, Fig. 2c). Compared with E- plants, E+ plants had more seeds per inflorescence and higher seed yields per plant under both salt stressed conditions, but the difference was only significant under HS stress conditions, where seed numbers per inflorescence of E+ plants were increased by 51% and seed yields by 158% (Fig. 2d, e). The thousand kernel weights did not differ significantly between E+ and E- plants. However, more remarkable, E+ plants produced a higher thousand kernel weight than E- plants under two salt stress conditions (Fig. 2f). Endophyte infection strongly increased the yield under high salt stress (Fig. 2, Table1).

Changes of physiological parameter

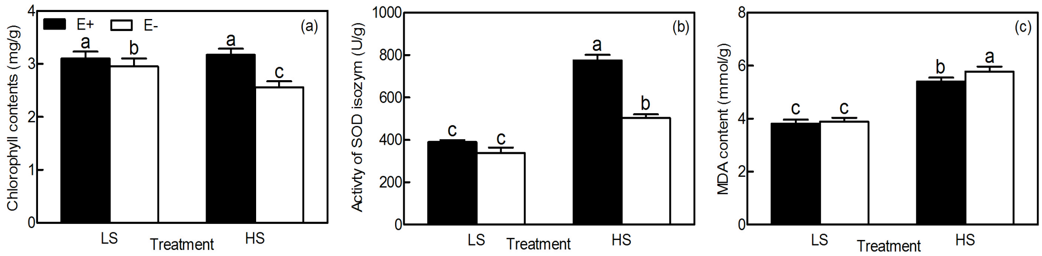

Fig. 3 Chlorophyll content (a), activity of Superoxide dismutase (SOD) (b), malondialdehyde (MDA) content (c), of *H. brevisubulatum* per plant as affected by endophyte infection (E+ and E-) and high (HS) or low salt (LS) content (Values are Means ±SE). Different letters indicate significant at $p < 0.05$ among treatments (Tukey test).

Among Changes of physiological parameter, endophyte infection increased chlorophyll content, superoxide dismutase (SOD) activity and decreased Malondialdehyde (MDA) content (Fig. 3c) of plants when the wild barley was under high salt stress (HS). However, it is worth noting that, for all treatments, E+ plants had higher chlorophyll content (Fig. 3a) and superoxide dismutase (SOD) activity (Fig. 3b) than E- plants. Significant effects of endophyte were detected chlorophyll content, SOD enzyme activity and malondialdehyde content in plants (Table 1).

Endophyte enhances seed germination under salt stress

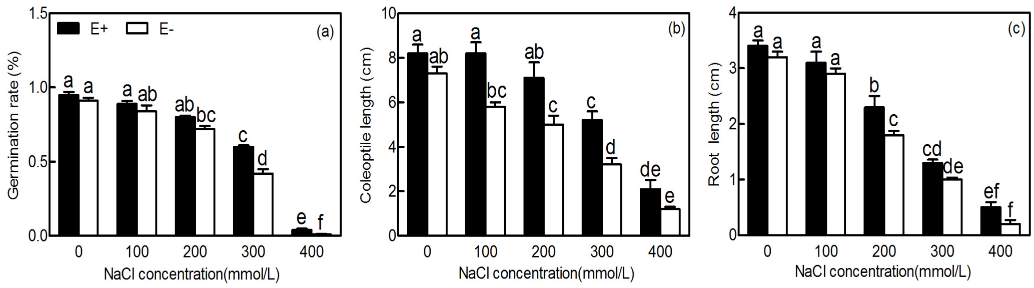

Fig.4 Germination rate (a), coleoptile length (b) and root length (c) of H. brevisubulatum per plant as affected by endophyte infection (E+ and E-) and treated with different NaCl content (values are Means ± SE). Different letters indicate significant at $p < 0.05$ among treatments (Tukey test).

The endophyte had significant effects on germination rate, root lengths and coleoptile lengths (Table 1). Germination rate decreased with increasing NaCl concentration, and there were significant differences in E+ and E- plants under NaCl concentrations 300 and 400 mM (Fig. 4a). Similarly, coleoptile lengths and root lengths were significantly greater for E+ than E- seedlings when the NaCl concentration was 200 mM (Fig. 4b, c).

Table 1 Two-way ANOVA for the endophyte status (E), salt treatment (S) and the interaction E × S for the parameters of tillers per plant, plant height, relative tillering rate, total dry weight per plant, ratio of reproductive tillers, inflorescences per plant, length of inflorescences, seeds number per inflorescence, seed yield per plant, thousand kernel weight, chlorophyll content, SOD activity, MDA content, germination rate, coleoptile length and root length variables of *H. brevisubulatum.*

Parameter	Effect	df	F-value	P
Tillers per plant	E	1,68	1.16	0.284
	S	1,68	0.19	0.662
	E × S	1,68	0.02	0.880
Plant height (cm)	E	1,68	6.34	**0.014**
	S	1,68	0.71	0.401
	E × S	1,68	0.71	0.401
Relative tillering rate (day^{-1})	E	1,20	9.58	**0.006**
	S	1,20	0.41	0.529
	E × S	1,20	3.69	0.069
Total dry weight per plant (g)	E	1,20	27.09	**<0.001**
	S	1,20	117.67	**<0.001**
	E × S	1,20	9.74	**0.005**
Ratio of reproductive tillers (%) *	E	1,20	47.59	**<0.001**
	S	1,20	131.98	**<0.001**
	E × S	1,20	105.70	**<0.001**
Inflorescences per plant	E	1,20	21.30	**<0.001**
	S	1,20	21.30	**<0.001**
	E × S	1,20	15.65	**0.001**
Length of inflorescences (cm)	E	1,20	31.29	**<0.001**
	S	1,20	41.66	**<0.001**
	E× S	1,20	0.18	0.672
Seed number per inflorescence	E	1,20	22.88	**<0.001**
	S	1,20	6.75	**0.017**
	E× S	1,20	0.84	0.369

Seed yield per plant (g)	E	1,20	11.05	**0.003**
	S	1,20	3.67	0.070
	E× S	1,20	16.36	**0.001**
Thousand kernel weight (g)	E	1,20	0.78	0.386
	S	1,20	3.88	0.063
	E × S	1,20	0.003	0.957
Chlorophyll content (mg/g FW)	E	1,20	135.80	**<0.001**
	S	1,20	25.91	**<0.001**
	E × S	1,20	54.23	**<0.001**
SOD activity (U/g FW)	E	1,20	62.79	**<0.001**
	S	1,20	182.69	**<0.001**
	E × S	1,20	28.42	**<0.001**
MDA content (umol/g FW)	E	1,20	36.70	**<0.001**
	S	1,20	6.11	**<0.001**
	E × S	1,20	83.66	**<0.001**
Germination rate (%) *	E	1,50	645.70	**<0.001**
	S	4,50	44.37	**<0.001**
	E × S	4,50	7.21	**<0.001**
Coleoptile length (cm)	E	1,50	45.31	**<0.001**
	S	4,50	79.96	**<0.001**
	E × S	4,50	1.72	0.159
Root length (cm)	E	1,50	16.93	**<0.001**
	S	4,50	168.22	**<0.001**
	E × S	4,50	1.48	0.221

* Arcsine square root was used to transform the ratio of reproductive tiller and germination rate.

Discussion

Endophytes and Stress Tolerance

In the study of E+ and E- plants grown in LS and HS treatments, we found that endophyte infection promotes plant growth, physiological changes and seed germination under HS conditions. A large number of studies have shown that endophytes promote plant growth under abiotic environmental stress (Cheplick et al. 1989; Morse et al. 2002; Hesse et al. 2003; Vila-Alub et al. 2005; Rahman et al. 2005; Kumkum et al. 2015). In this research, the results obtained for wild barley (from the high saline site) suggested that endophyte infected plants can grow normally under HS stress. We found that under certain salt stress conditions, endophyte infection can improve the growth and seed germination of wild barley. That is, endophyte infection seemed to modify salt-induced inhibition of plant growth.

Increasing Forage Crop Yields

This study has shown that *Epichloë* endophyte infection in wild barley increases forage crop yields as indicated by E+ plants that produce more tillers and taller plants, than E- plants under HS stress treatment. Although the dry weights of vegetative tillers were higher for E+ plants under both salt stress condition compared with E- plants, the differences were not significant. These investigations illustrate that endophytes reduce inhibition of plant growth due to salt stress. Similar research findings indicated that fungal endophytes isolated from plants in saline soils have the potential to confer saline tolerance to crop plants in arid environments (Kumkum et al. 2015).

Endophyte-Mediated Reduction of Salt Stress Inhibition of Reproductive Growth

To determine whether the plants reproductive growth was affected by endophyte infection and salt stress, we measured forage crop yield parameters. Results of this study confirmed that E+ plants showed increased numbers of reproductive tillers, inflorescences per plant, and increased seed yields per plant, compared with E- plants in the HS treatment. In addition E+ plant inflorescences were significantly longer than those of E- plants under both salt treatments. Contrary to previous reports on perennial ryegrass (Latch et al. 1985; Hesse et al. 2003), endophyte infection did not affect the individual seed weight. Thus, this is clear evidence that endophyte infection increased wild barley crop yields under salt stress conditions.

Chlorophyll Content as an Indicator of Stress Tolerance

Understanding the physiological reactions of *H. brevisubulatum* selections under soil salt stress is essential to establishment of saline tolerant breeding populations. A number of studies have shown that reduced photosynthesis is one of the main factors that lead to decreased plant growth and production (Ashraf 2004; Dubey 2005). In this study, chlorophyll content was significantly lower in E- plants than in E+ plants in both salt stress treatments. Plant chlorophyll content was significantly affected by endophytes and endophyte × salt stress interactions. It is suggested that the increased chlorophyll content of E+ plants under HS stress is a result of increased tolerance to the high salt environment. These results were consistent with findings by Cheng et al (2018a), who found *Epichloë* infected *H. brevisubulatum* plants also demonstrated improved photosynthesis under salt stress and alkali stress.

Protection from Oxidative Stress

Increase in superoxide dismutase (SOD) activity is one of the factors for cell resistance to reactive oxygen, and its activity can reflect the strength of plant resistance. Results reported here showed that the activity of SOD in E- plants was lower than that in E+ plants under high salt stress, and significantly affected only by endophyte status. These results may suggest that endophytic fungi confer salt resistance to plants by stimulating the activity of SOD, and making plants more resistant to affects of reactive oxygen that results from stress. A previous study revealed that *Achnatherum inebrians* responds to salt stress by increasing the activity of SOD (Zhang and Nan. 2007) and active nodule colonization increases salt tolerance to alfalfa by increasing the activity of antioxidant enzymes (Wang et al. 2016).

The stability of cell membranes can be used to determine resistance of plants to oxidative stress, and the content of malondialdehyde (MDA) resulting from membrane peroxidation is an important index for strength of membranes. The content of MDA from oxidation of membrane lipids was lower in plants with endophytes and this correlated with higher salt stress resistance. Endophytes significantly affected the MDA content of *H. brevisubulatum*. The endophytic fungus can effectively protect the membrane system of *H. brevisubulatum*, thus improving the salinity tolerance of E+ plants. Previous studies reported that *M. sativa* plants infected with fungus *P. indica* had lower MDA content under high salt concentration (Li et al. 2017). Results reported here suggest that plant response to antioxidant endophytic fungi increases survival ability of *H. brevisubulatum* and better adapts plants to high salt soils.

Stimulation of Seed Germination

Seed germination is important because it is the beginning of plant life cycles (Weitbrecht et al. 2011). In this paper, the *Epichloë* endophyte of wild barley is spread mainly by seeds, and thus it seems logical that the endophyte affects seed germination, seedling growth and a series of host life activities. The seed experiments indicate that the endophyte affects on germination of *H. brevisubulatum* seeds is determined by NaCl concentration. When the seeds were in 0 and 100 mM NaCl, there were no significant effects on germination parameters (germination rate, coleoptile length and root length) resulting from endophyte infection, but at higher NaCl concentrations (200 and 300 mM), the endophyte significantly increased root length, coleoptile length and germination rate. This result showed that endophytic infection of plants benefited seedling growth; this advantage to the host plants is more obvious under high salt stress. Endophyte infection has been found to increase germination in *Schedonorus arundinaceus* (Pinkerton et al. 1990), *B. setifolius* (Novas et al. 2003), *F. rubra* (Wäli et al. 2009), *L. perenne* (Ma et al. 2015) and *Phyllanthus amarus* (Joe et al. 2016).

Conclusions

Results reported here demonstrate that the *Epichloë* endophyte wild barley had a positive effect on salt tolerance of its host by improving germination and growth capacity. These results also confirm that growth conditions of the original habitat may affect the symbiotic relationship between plants and fungi, and wild barley could be adapted to salt environments by natural selection, with the E+ ecotype being more tolerant to salt than the E- ecotype. The *Epichloë* endophyte of wild barley appears to function in adapting plants to the environment, in this case, high salinity soils. These findings suggest that the endophyte in wild barley has potential to play a critical role in improving salinity tolerance, in plants designed for growth in alkalized meadows and other saline-alkali lands.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflicts of interest.

References

Ashraf M, Harris PJC (2003) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166:3-16. doi:10.1016/j.plantsci.2003.10.024

Azevedo MD, Welty RE (1995) A study of the fungal endophyte *Acremonium coenophialum* in the roots of tall fescue seedlings. Mycologia 87:289-97. doi:10.2307/3760825

Chen TX, Johnson R, Chen SH, Lv H, Zhou JL, Li CJ (2018a) Infection by the fungal endophyte *Epichloë bromicola* enhances the tolerance of wild barley (*Hordeum brevisubulatum*) to salt and alkali stresses. Plant Soil 428:353-370. doi:10.1007/s11104-018-3643-4

Chen TX, Simpson RW, Song QY, Chen SH, Li CJ, Ahmad ZR (2018b) Identification of *Epichloë* endophytes associated with wild barley (*Hordeum brevisubulatum*) and characterisation of their alkaloid biosynthesis. New Zeal J Agr Res doi:10.1080/00288233.2018.1461658

Cheplick GP, Clay K, Marks S (1989) Interactions between infection by endophytic fungi and nutrient limitation in the grasses *Lolium perenne* and *Festuca arundinacea*. New Phytol 111:89-97. doi:10.1111/j.1469-8137.1989.tb04222.x

Cheplick GP, Perera A, Koulouris K (2000) Effect of drought on the growth of *Lolium perenne* genotypes with and without fungal endophytes. Funct Ecol 14:657-667. doi:10.1046/j.1365-2435.2000.00466.x

Dubey RS (2005) Photosynthesis in plants under stressful conditions. In: Pessarakli, M. editor. Handbook of photosynthesis. 2nd ed. Florida, CRC Press. pp. 479-497

Eerens JPJ, Lucas RJ, Easton HS, White JGH (1998) Influence of the ryegrass endophyte (*Neotyphodium lolii*) in a cool-moist environment II. Sheep production. New Zeal J Agr Res. 41:191-199. doi:191-199.10.1080/00288233.1998.9513286

Faeth SH, Helander ML, Saikkonen KT (2004) Asexual *Neotyphodium* endophytes in a native grass reduce competitive abilities. Ecol Lett 7:304-313. doi:10.1111/j.1461-0248.2004.00578.x

Fang SZ, Song LY, Fang XX (2006) Effects of NaCl stress on seed germination, leaf gas exchange and seedling growth of *Pteroceltis tatarinowii*. Journal of Forestry Research 17(3):185-188. doi:10.1007/s11434-006-0723-2

Gou XY (2007) Effects of NaCl stress on growth and physiological characteristics of *Neotyphodium* endophyte infected and free *Achnatherum inebrians.* Master thesis. Lanzhou University.

Hesse U, SchÖberlein W, Wittenmayer L, Förster K, Warnstorff K, Diepenbrock W, Merbach W (2003) Effects of *Neotyphodium* endophytes on growth, reproduction and drought-stress tolerance of three *Lolium perenne* L. genotypes. Grass For Sci 58:407-415. doi:10.1111/j.1365-2494.2003.00393.x

Jia SX (1987) Flora of Forages in China (The First Volume). Beijing: Agriculture Press 121-124

Joe MM, Devaraja S, Benson A, Sa T (2016) Isolation of phosphate solubilizing endophytic bacteria from *Phyllanthus amarus* Schum & Thonn: Evaluation of plant growth promotion and antioxidant activity under salt stress. J. Appl. Res. Med. Aromat. Plants. http://dx.doi.org/10.1016/j.jarmap.2016.02.003

Kimmons CA, Gwinn KD, Bernard EC (1990) Nematode reproduction on endophyte-infected and endophyte-free tall fescue. Plant Dis 74:757-761. doi:10.1071/APP9930040

Kumkum A, Susan K (2015) A fungal endophyte strategy for mitigating the effect of salt and drought stress on plant growth. Symbiosis 68:73-78. doi:10.1007/s13199-015-0370-y

Latch GCM, Hunt WF, Musgrave DR (1985) Endophytic fungi affect growth of perennial ryegrass. New Zeal J Agr Res 28:165-168. doi:org/10.1080/00288233.1985.10427011

Li CJ, Nan ZB, Gao JH, Tian P (2004) Detection and distribution of *Neotyphodium-Achnatherum inebrians* association in China. Proceedings of 5th international *Neotyphodium*/grass interactions symposium, Arkansas.

Li CJ, Nan ZB, Liu Y, Paul VH, Peter D (2008) Methodology of endophyte detection of drunken horse grass (*Achnatherum inebriants*). Edible Fungi of China 27:16-19

Li L, Li L, Wang XY, Zhu PY, Wu HQ, Qi ST (2017) Plant growth-promoting endophyte *Piriformospora indica* alleviates salinity stress in *Medicago truncatula.* Plant Physiol Bioch 119:211-223. doi:org/10.1016/j.plaphy.2017.08.029

Ma MZ, Christensen MJ, Nan ZB (2015) Effects of the endophyte *Epichloë festucae* var. *lolii* of perennial ryegrass (*Lolium perenne*) on indicators of oxidative stress from pathogenic fungi during seed germination and seedling growth. Eur J Plant Pathol 141:571-583 doi:10.1007/s10658-014-0563-x

Malinowski DP, Belesky DP, Lewis GC (2005a) Abiotic stresses in endophytic grasses. In: *Neotyphodium in Cool-Season Grasses* (Eds Roberts CA, West CP, Spiers DE), Blackwell Publishing Professional, Ames, Iowa, USA 187-199

Morse LJ, Day TA, Faeth SH (2002) Effect of *Neotyphodium* endophyte infection on growth and leaf gas exchange of Arizona fescue under contrasting water availability regimes. Environ Exp Bot 48:257-268. doi:org/10.1016/S0098-8472(02)00042-4

Muscolo A, Sidari M, Panuccio MR (2003) Tolerance of kikuyu grass to long term salt stress is associated with induction of antioxidant defences. Plant Growth Regul 41:57-62. doi:10.1023/A:1027378417559

Nan ZB, Li CJ (2000) *Neotyphodium* in native grasses in China and observations on endophyte/host interactions. Proceedings of the 4th International *Neotyphodium*/Grass Interactions Symposium, Soest, Germany. pp. 41-50

Novas MV, Gentile A, Cabral D (2003) Comparative study of growth parameters on diaspores and seedlings between populations of *Bromus setifolius* from Patagonia, differing in *Neotyphodium* endophyte infection. Flora 198:421-426. doi:org/10.1078/0367-2530-00115

Pinkerton BW, Rice JS, Undersander DJ (1990) Germination in *Festuca arundinacea* as affected by the fungal endophyte, *Acremonium coenophialum*. In: Proceedings of the International Symposium: On *Acremonium*/Grass Interactions (eds S. S. Quinsenberry & R. E. Joost) New Orleans, pp 176

Rahman MH, Saiga S (2005) Endophytic fungi (*Neotyphodium coenophialum*) affect the growth and mineral uptake, transport and efficiency ratios in tall fescue (*Festuca arundinacea*). Plant Soil 272:163-171. doi:10.1007/s11104-004-4682-6

Redman RS, Kim YO, Woodward CJD, Greer C, Espino L, Doty SL, Rodriguez RJ (2011) Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. PloS One 6(7):e14823. doi:10.1371/journal.pone.0014823

Ren AZ, Gao YB, Zhang J, Zhang J (2006) Effect of endophyte infection on salt resistance of ryegrass. Acta Ecol Sin 26(6):1750-1757

Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314-330. doi:10.1111/j.1469-8137.2009.027773.x

Seto Y, Takahashi K, Matsuura H, Kogami Y, Yada H, Yoshihara T, Nabeta K (2007) Novel cyclic peptide, epichlicin, from the endophytic fungus, *Epichloë typhina*. Biosci Biotechnol Biochem 71(6):1470-1475. doi: 10.1271/bbb.60700

Shrivastava P, Kumar R (2015) Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22:123-131. doi:org/10.1016/j.sjbs.2014.12.001

Song ML, Chai Q, Li XZ, Yao X, Li CJ, Christensen M J, Nan ZB (2015) An asexual *Epichloë* endophyte modifies the nutrient stoichiometry of wild barley (*Hordeum brevisubulatum*) under salt stress. Plant Soil 387(1):153-165. doi:10.1007/s11104-014-2289-0

Vila-Alub MM, Gundel PP, Ghersa CM (2005) Fungal endophyte infection changes growth attributes in *Lolium multiflorum* Lam. Austral Ecol 30:49-57. doi:10.1007/s11104-014-2289-0

Wang JJ, Zhou YP, Lin WH, Li MM, Wang MN, Wang ZG, Kuang Y, Tian P (2017) Effect of an *Epichloë* endophyte on adaptability to water stress in *Festuca sinensis.* Fungal Ecol 30:39-47. doi: 10.1016/j.funeco.2017.08.005

Wang P (2003) The study of ecology of pasture sown- wild barley. Master thesis. Northeast Normal University.

Wang ZF (2009) Effect of *Neotyphodium* Endophyte Infection on Salt Tolerance of *Hordeum brevisubulatum*. Master thesis: Lanzhou University.

Wang ZW, Wang SM, Ji YL, Zhao MW, Yu HW (2005) Plant endophyte research 6: Detection and distribution of endophytic fungus in Gramineous plants in saline alkali area in Dongying. Pratacul

Sci 2:60-64

Wang YF, Zhang ZQ, Zhang P, Cao YM, Hu TM, Yang PZ (2016) Rhizobium symbiosis contribution to short-term salt stress tolerance in alfalfa *(Medicago sativa* L.). Plant Soil 402:247-261. doi:10.1007/s11104-016-2792-6

Wäli PR, Helander M, Saloniemi I, Ahlholm J, Saikkonen K (2009) Variable effects of endophytic fungus on seedling establishment of fine fescues. Oecologia 159:49-57. doi:10.1007/s004442-008-1202-z

Weitbrecht K, Muller K, Leubner-Metzger G (2011) First off the mark: early seed germination. J Exp Bot 62:289-3309. doi:10.1093/jxb/err030

Yang T, Chen Y, Wang X, Dai C (2013) Plant symbionts: keys to the phytosphere. Symbiosis 59:1-14. doi:10.1007/s13199-012-0190-2

Yang T, Ma S, Dai CC (2014) Drought degree constrains the beneficial effects of a fungal endophyte on *Atractylodes lancea*. J Appl Microbiol 117:1435-1449. doi:10.1111/jam.12615

Yu L, Wang YR, Song JH (1999) Studies on germination condition and stress resistance of *Hordeum brevisubulatum* seeds. Acta Prata Sin 3:50-57

Zabalgogeazcoa I, Romo M, Keck E, Vázquez de Aldana BR, García Ciudad A, García Criado B (2006) The infection of *Festuca rubra* subsp. *pruinosa* by *Epichloë festucae*. Grass For Sci 61:71-76. doi:10.1111/j.1365-2494.2006.00509.x

Zhang YP, Nan ZB (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. doi:10.1111/j.1439-037X.2007.00279.x