

SALINITY STRESS

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

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Abstract

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. In this research, we determined the effects of the *E. bromicola* endophyte on growth, physiological properties and seed germination of wild barley under salt stress through a set of experiments. Our results demonstrate that endophyte-infected (E+) plants produced more tillers, higher biomass and yield, higher chlorophyll content and superoxide dismutase activity than endophyte-free (E-) plants under high salt stress. Seed germination parameters of E+ biotype were significantly higher than those of E- plants when NaCl concentration reached 200 and 300 mM. Our results demonstrate that *E. bromicola* endophytes increased tolerance to salt stress in wild barley by increasing seed germination and growth, and altering plant physiology.

KEYWORDS

biomass, *Epichloë bromicola*, *Hordeum brevisubulatum*, saline stress, seed yield

1 | 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, Sidari, & Panuccio, 2003; Shrivastava & 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 and has been widely cultivated in Jilin, Inner Mongolia, Hebei, Gansu, Qinghai and Xinjiang provinces of China (Jia, 1987). In addition, numerous wild

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

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 bromicola 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 (Chen, Li, White, & Nan, 2018; Li, Nan, Gao, & Tian, 2004; Malinowski, Belesky, & Lewis, 2005; Seto et al., 2007; Wang, Helander, Saloniemi, Ahlholm, & Saikkonen, 2009). Some fungal endophytes facilitate plant growth even in harsh environments (Rodriguez, White, Arnold, & Redman, 2009; Yang, Chen, Wang, & Dai, 2013). Studies have reported that endophyte presence affects plant growth, increasing tiller number, biomass production and seed yield (Azevedo & Welty, 1995; Hesse et al., 2003; Kimmons, Gwinn, & Bernard, 1990; Vila-Alub, Gundel, & Ghersa, 2005). However, not all endophytes show the same benefits in hosts under all conditions. For instance, Faeth, Helander, and Saikkonen (2004) found that endophytes did not affect the seed germination of Arizona fescue (*Festuca arizonica* Vasey [Pooideae]), and Eerens, Lucas, Easton, and White (1998) and Cheplick, Perera, and Koulouris (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, Gao, Zhang, & Zhang, 2006) and *H. brevisubulatum* infected with *E. bromicola* had faster seedling growth under salt stress (Song et al., 2015). However, endophytes may also decrease salt tolerance in their hosts. For instance, the *Epichloë* endophyte frequency in a population of *Roegneria* samples from a saline-alkali area decreased as the concentration of saline-alkali in the sampling zone increased in Dongying, Shandong Province, China (Wang et al., 2016). One study indicated that endophytes did not influence biomass production of *Festuca rubra* under soil salinity conditions (Zabalgogezcoa 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 (Azevedo & Welty, 1995; Kimmons et al., 1990). 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 (Hesse et al., 2003; Vila-Alub et al., 2005). To evaluate the hardiness of plants under salt stress, plants may be assessed for chlorophyll content, superoxide dismutase (SOD) activity and malondialdehyde (MDA) content (Hamilton, Gundel, Helander, & Saikkonen, 2012). 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 (Weitbrecht, Muller, & Leubner-Metzger, 2011).

Epichloë bromicola endophyte infection of *H. brevisubulatum* plants in 13 populations in China has been reported to range from 67% to 100% (Nan & Li, 2000; Wang, 2017). Several researchers

have investigated the physiological function of *E. bromicola* endophyte infection in *H. brevisubulatum* plants under soil salt stress, and studies have shown that *E. bromicola* endophytes could enhance salt tolerance (Chen, Johnson, et al., 2018; Chen, Li, et al., 2018; Song et al., 2015; Wang, 2017). However, it is still unknown how the *E. bromicola* endophyte in *H. brevisubulatum* plants affects forage yield, seed output and new generation seed germination under saline field conditions. Thus, to expand our understanding of the breeding of *H. 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.

2 | MATERIALS AND METHODS

2.1 | Plant material

Hordeum brevisubulatum seeds were collected from wild plants in August 2008 from Linze (Altitude: 1,580 m, E: 100°06', N: 39°11'), Zhangye City, Gansu Province, China. Seeds were assessed for *E. bromicola* endophyte infection through microscopic examination using stain 0.8% aniline blue (Chen et al., 2019; Li et al., 2004). Of the 100 seeds evaluated, 100% were found to be E+. To obtain E- plant material, half of the E+ seeds (from one plant) of *E. bromicola* endophyte-infected wild barley were treated using benomyl fungicide for 1 hr to produce E- seeds (Cheplick, Clay, & Marks, 1989). To reduce impacts of the fungicide treatment, E- and E+ plants were grown in the field for 2 years. The infection status of all plants was confirmed by microscopic examination (Li, Nan, Liu, Paul, & Peter, 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 (15–25°C) with 14 hr of illumination (800 $\mu\text{mol m}^{-2} \text{s}^{-1}$) 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).

2.2 | Field experiment

Each experimental block (240 cm length, 240 cm width and 50 cm height) consisting of loess soil that had no history of growing crops was located in the College of Pastoral Agriculture Science and Technology (Altitude: 1,520 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 blocks with one of two salt stress: low (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 up a salt content of 3 g NaCl per 100 g soil]), using a Soil-EC Tester, Beijing, China. Each salt stress included two

types of endophyte treatments (i.e. E+ and E-). The experiment consisted of four plots with three sub-plots nested in each, and two rows of plants (10 plants per row) within each sub-plot. Both row distance and plant distance is 20 cm. To prevent salt penetration, each sub-plot was separated by concrete walls. 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 sub-plot at week 4, 8 or 16, and plant height and number of tillers per plant were measured (24 plants per salt concentration). The relative tillering rate (RTR) was calculated as $(\ln T_2 - \ln T_1) / 70d$, where T_1 is the tiller number at week 4 and T_2 is the tiller number at week 16, following Cheplick (1998). All plants were harvested on 20 August 2010 and grown for about 130 days. The total biomass was determined after being oven-dried at 60°C 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 (16 week).

2.3 | 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$) of Baiyin Agricultural Science Research Institute (altitude: 1,702 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 \times 2 endophyte infection status types \times 6 replicates (pots) \times 5 plants per pot (18 cm in diameter \times 16 cm in height), resulting in a total of 120 plants. Eight weeks after the start of experiment, we determined chlorophyll content, MDA concentration and 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 per plot was extracted in 80% acetone and centrifuged at 4,000 rcf 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. SOD activity was detected in leaf tissue (0.05 g in FW) as described by Giannopolitis and Ries (1977). MDA content was determined according to the thio-barbituric acid (TBA) reaction method (Puckette, Weng, & Mahalingam, 2007) using a spectrophotometer (SP-723). The sample of the supernatant liquid was measured at 450, 532 and 600 nm wavelengths, and then, the malondialdehyde concentration was calculated using the following formula: $C = 6.45(A_{532} - A_{600}) - 0.56A_{450}$. MDA concentration was expressed as millimoles per gram fresh weight (FW).

2.4 | Seed germination experiment

Seeds of wild barley were collected from E+ and E- plants that grew in the experimental field. Seeds were dried in natural sunlight and

then 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 tested in a germination chamber with alternating temperatures of 15/25°C (12 hr a.m./12 hr p.m.) and with a 80% relative humidity. The humidity and temperature conditions are optimal for wild barley germination (Yu, Wang, & Song, 1999). Seeds were germinated on filter paper in Petri dishes (diameter = 15 cm) involving 5 salt concentrations \times 2 endophyte types \times 6 replicates per concentration \times 100 seeds per replicate, resulting in a total of 6,000 seeds used. The numbers of germinated seeds were counted, and the lengths of the roots and coleoptiles were measured at the end of the study (after 14 days). This was done according to the International Seed Testing Association (ISTA) protocols (ISTA 1999).

2.5 | Statistical analysis

All analyses were performed using SPSS version 19.0 software (SPSS Inc). Prior to ANOVA, data were checked for normality and homogeneity and were transformed (ratio of reproductive tillers and germination rate) 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), germination rate, coleoptile length and root length were analysed 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 ($p < .05$).

3 | RESULTS

3.1 | Endophyte enhanced growth under salt stress

The plant height and tiller number of E+ plants were greater than that of E- plants under two salt stress levels at 4, 8 and 16 weeks after treatments (Figure 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 ($p < .05$, Figure 1a). E+ plants had approximately 41% ($p < .05$) higher RTR and 25% ($p < .05$) 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 ($p > .05$, Figure 1c,d). RTR and total dry weight of biomass per plant were significantly affected by endophyte infection ($p < .01$, $p < .001$, respectively, Table 1). For total dry weight of biomass, the endophyte \times salt interaction was also significant ($p < .05$, Table 1). These results indicate that the effect of *E. bromicola* endophyte on plant growth was affected by salt stress level.

3.2 | Endophyte enhanced yield under salt stress

Endophyte infection significantly increased the ratio of reproductive tillers to non-reproductive tillers by 116% ($p < .05$) in the HS-stressed

TABLE 1 Two-way ANOVA for the *Epichloë bromicola* endophyte status (E), salt treatment (S) and the interaction E × S for the parameters of tillers per plant, plant height, RTR, total dry weight per plant, ratio of reproductive tillers, inflorescences per plant, length of inflorescences, seed number per inflorescence, seed yield per plant, TKW, chlorophyll content, SOD activity, MDA content, germination rate, coleoptile length and root length variables of *Hordeum brevisubulatum*

Parameter	Effect	df	F-value	p
Tillers per plant	E	1, 68	1.16	.284
	S	1, 68	0.19	.662
	E × S	1, 68	0.02	.880
Plant height (cm)	E	1, 68	6.34	.014
	S	1, 68	0.71	.401
	E × S	1, 68	0.71	.401
RTR (/day)	E	1, 20	9.58	.006
	S	1, 20	0.41	.529
	E × S	1, 20	3.69	.069
Total dry weight per plant (g)	E	1, 20	27.09	<.001
	S	1, 20	117.67	<.001
	E × S	1, 20	9.74	.005
Ratio of reproductive tillers (%) ^a	E	1, 20	47.59	<.001
	S	1, 20	131.98	<.001
	E × S	1, 20	105.70	<.001
Inflorescences per plant	E	1, 20	21.30	<.001
	S	1, 20	21.30	<.001
	E × S	1, 20	15.65	.001
Length of inflorescences (cm)	E	1, 20	31.29	<.001
	S	1, 20	41.66	<.001
	E × S	1, 20	0.18	.672
Seed number per inflorescence	E	1, 20	22.88	<.001
	S	1, 20	6.75	.017
	E × S	1, 20	0.84	.369
Seed yield per plant (g)	E	1, 20	11.05	.003
	S	1, 20	3.67	.070
	E × S	1, 20	16.36	.001
TKW (g)	E	1, 20	0.78	.386
	S	1, 20	3.88	.063
	E × S	1, 20	0.003	.957
Chlorophyll content (mg/g FW)	E	1, 20	135.80	<.001
	S	1, 20	25.91	<.001
	E × S	1, 20	54.23	<.001
SOD activity (U/g FW)	E	1, 20	62.79	<.001
	S	1, 20	182.69	<.001
	E × S	1, 20	28.42	<.001
MDA content (umol/g FW)	E	1, 20	36.70	<.001
	S	1, 20	6.11	<.001
	E × S	1, 20	83.66	<.001

(Continues)

TABLE 1 (Continued)

Parameter	Effect	df	F-value	p
Germination rate (%) ^a	E	1, 50	645.70	<.001
	S	4, 50	44.37	<.001
	E × S	4, 50	7.21	<.001
Coleoptile length (cm)	E	1, 50	45.31	<.001
	S	4, 50	79.96	<.001
	E × S	4, 50	1.72	.159
Root length (cm)	E	1, 50	16.93	<.001
	S	4, 50	168.22	<.001
	E × S	4, 50	1.48	.221

Note: Plant height and tillers per plant were measured at weeks 4, 8 or 16; the time factor has three levels: 4, 8 and 16 weeks after NaCl treatment; total dry weight per plant, ratio of reproductive tillers, inflorescences per plant, length of inflorescences, seed number per inflorescence, seed yield per plant and TKW were measured at 16 week; eight weeks after the start of pot experiment, chlorophyll content, MDA concentration and SOD activities of the E+ and E- plants were determined; field and pot study under two different salinity conditions: 0.03% and 3% NaCl; germination and seedling growth under 5 different salinity conditions: 0, 100, 200, 300, 400 mM NaCl; the *E. bromicola* factor has two levels: uninfected and *E. bromicola* infected; df, degrees of freedom; TKW, thousand-kernel weight; Significant ($p < .05$) F-values are indicated by bold characters.

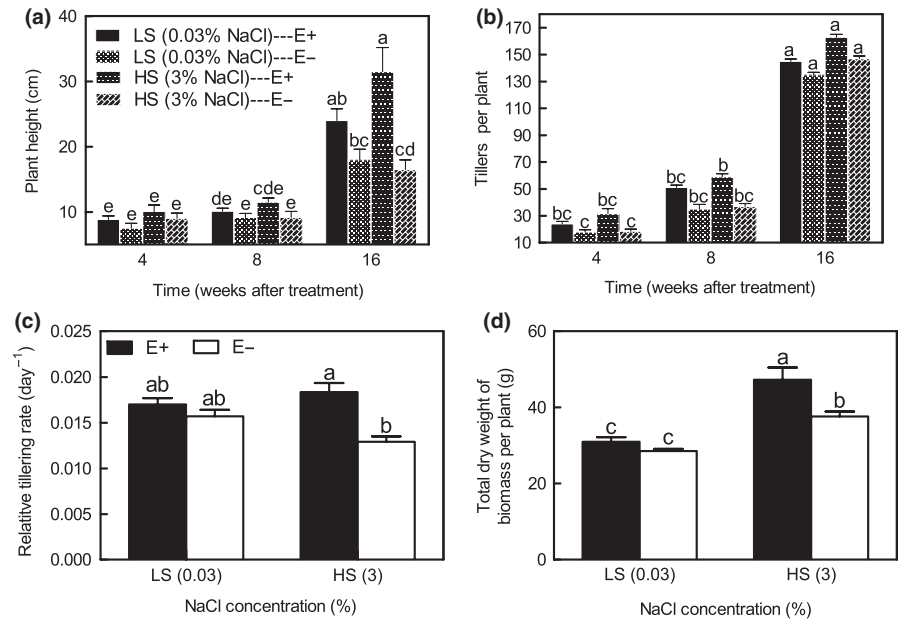
^aArcsine square root was used to transform the ratio of reproductive tiller and germination rate.

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 ($p > .05$, Figure 2a). The results indicated that E+ plants had 43% more inflorescences per plant than E- plants in the HS stress level ($p < .05$, Figure 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, $p < .05$, Figure 2c). Compared with E- plants, E+ plants had more seeds per inflorescence and higher seed yield per plant under both salt-stressed conditions, but the difference was only significant under HS stress conditions, where seed number per inflorescence of E+ plants was increased by 51% ($p < .05$) and seed yield by 158% ($p < .05$) (Figure 2d,e). The TKW did not differ significantly between E+ and E- plants. However, more remarkable, E+ plants produced a higher TKW than E- plants under two salt stress conditions (Figure 2f). Endophyte infection strongly increased the yield under high salt stress (Figure 2 and Table 1).

3.3 | Changes of physiological parameters

Among changes of physiological parameters, endophyte infection increased chlorophyll content, SOD activity and decreased MDA content ($p < .05$, Figure 3) of plants when the wild barley was under high salt stress (HS). However, it is worth noting that, for high salt treatment, E+ plants had higher chlorophyll content (Figure 3a) and SOD activity (Figure 3b) than E- plants. Significant effects of endophyte were detected in chlorophyll content, SOD enzyme activity and MDA content in plants ($p < .001$, Table 1).

FIGURE 1 Plant height (a), tillers per plant (b), relative tillering rate (c) and total dry weight of biomass per plant (d) of *Hordeum brevisubulatum* per plant as affected by endophyte infection (E+ and E-) and high (HS) or low (LS) salt content (values are means \pm SE). Different letters indicate significance at $p < .05$ among treatments (Tukey test)



3.4 | Endophyte enhances seed germination under salt stress

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 ($p < .05$) and 400 mM ($p < .05$) (Figure 4a). Similarly, coleoptile lengths were significantly greater for E+ than E- seedlings when the NaCl concentration was 100, 200 and 300 mM ($p < .05$, Figure 4b). Root lengths were significantly greater for E+ than E- seedlings when the NaCl concentration was 200 mM ($p < .05$, Figure 4c).

4 | DISCUSSION

4.1 | Endophytes and stress tolerance

In the study of E+ and E- plants grown in LS and HS treatments, we found that endophyte infection promoted 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; Dinkins, Nagabhyru, Young, West, & Schardl, 2019; Hesse et al., 2003; Kumkum & Susan, 2015; Morse, Day, & Faeth, 2002; Rahman & Saiga, 2005; Vila-Alub et al., 2005; Yang, Ma, & Dai, 2014). In this research, the results obtained for wild barley (from the high saline site) suggested that endophyte infection improved salinity stress tolerance. 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. These results indicate that *E. bromicola* could be used for the improvement of some characteristics of *H. brevisubulatum* cultivars used for reclamation of salt soils.

This study has shown that *E. bromicola* 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 trend higher for E+ plants under both salt stress conditions 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 enhance saline tolerance to crop plants in arid environments (Kumkum & Susan, 2015).

4.2 | Endophyte-mediated reduction of salt stress inhibition of reproductive growth

To determine whether the plant's 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 yield per plant, compared with E- plants in the HS treatment. These results are in agreement with previous studies that indicated that the *Epichloë amarillans*-infected *Agrostis hyemalis* plants produced more inflorescences and greater seeds mass than free plants at the lowest watering level, while at the lowest watering level, there were no significant difference between *E. amarillans*-infected plants and free plants in reproductive growth (Davitt, Chen, & Rudgers, 2011). In addition, E+ plant inflorescences were significantly longer than those of E- plants under both salt treatments. Contrary to previous reports on perennial ryegrass (Hesse et al., 2003; Latch, Hunt, & Musgrave, 1985) and *Ammophila breviligulata* (Emery, Bell-Dereske, & Rudgers, 2015), 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.

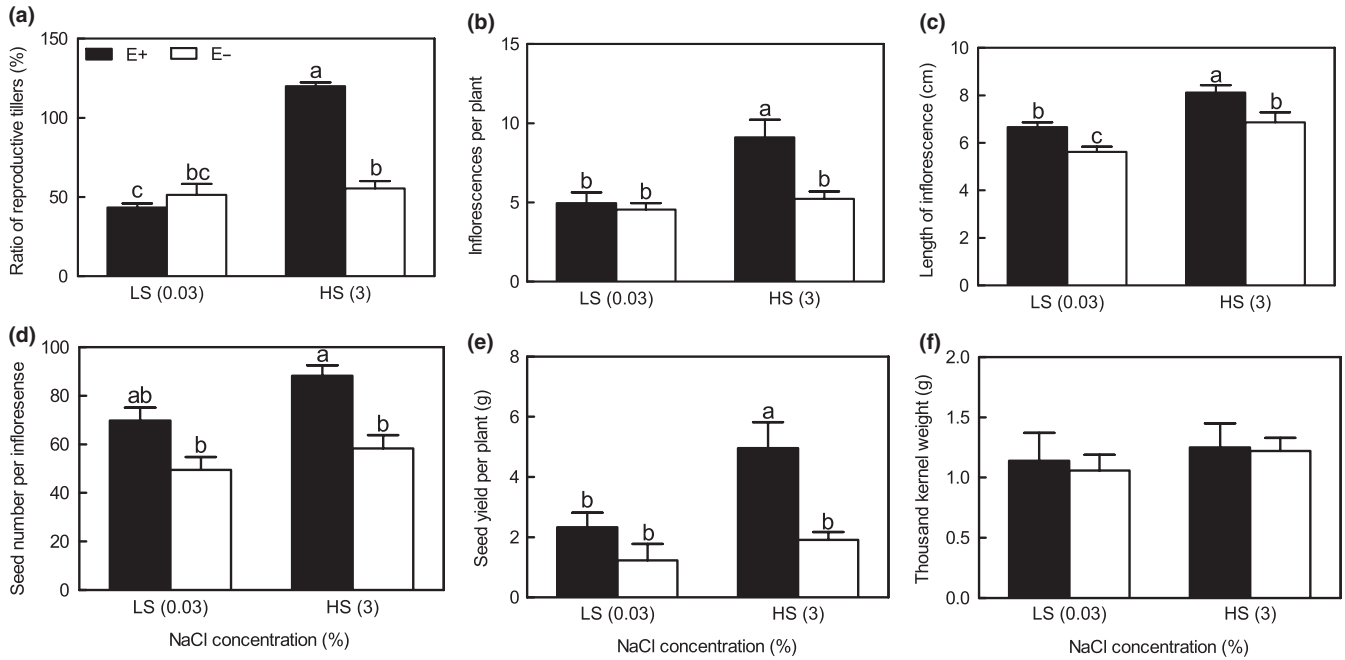


FIGURE 2 Ratio of reproductive tillers (a), inflorescences per plant (b), length of inflorescence (c), seed number per inflorescence (d), seed yield per plant (e) and thousand kernel weight (f) of *Hordeum brevisubulatum* per plant as affected by endophyte infection (E+ and E-) and high (HS) or low salt (LS) content (values are means \pm SE). Different letters indicate significance at $p < .05$ among treatments (Tukey test)

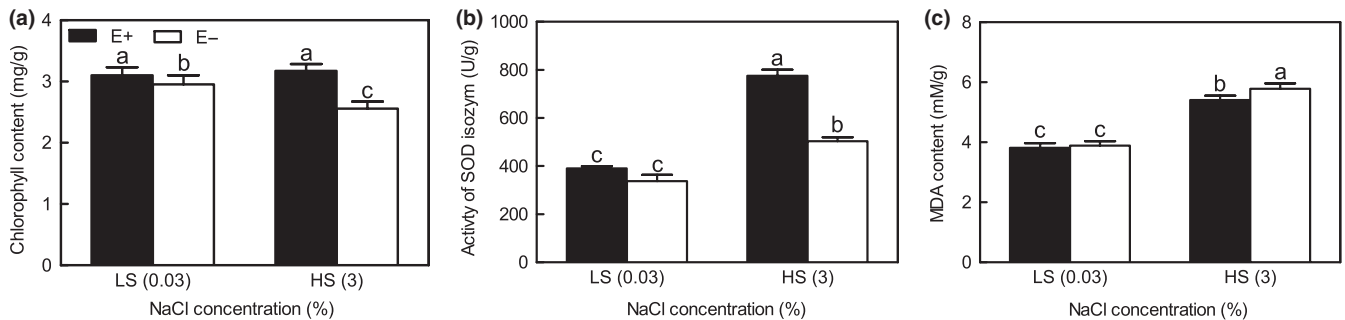


FIGURE 3 Chlorophyll content (a), activity of superoxide dismutase (SOD) (b), and malondialdehyde (MDA) content (c) of *Hordeum brevisubulatum* per plant as affected by endophyte infection (E+ and E-) and high (HS) or low salt (LS) content (values are means \pm SE). Different letters indicate significance at $p < .05$ among treatments (Tukey test)

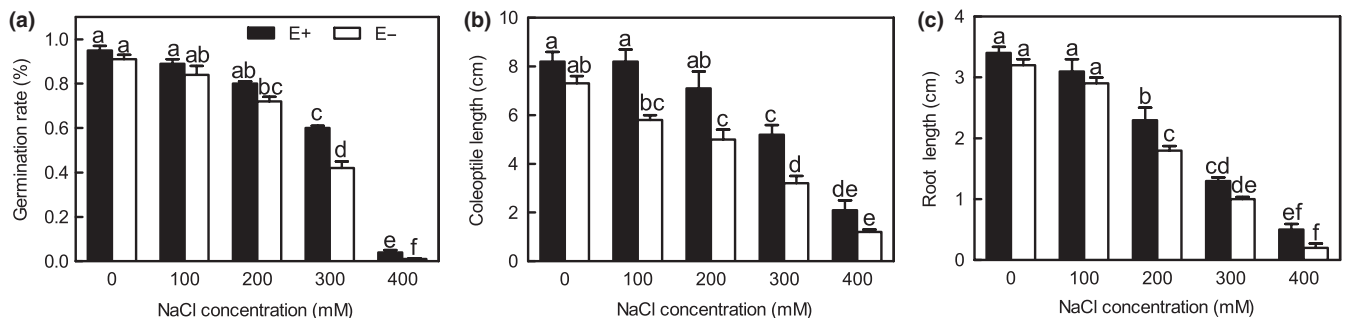


FIGURE 4 Germination rate (a), coleoptile length (b) and root length (c) of *Hordeum brevisubulatum* per plant as affected by endophyte infection (E+ and E-) and treated with different NaCl content (values are means \pm SE). Different letters indicate significance at $p < .05$ among treatments (Tukey test)

4.3 | Chlorophyll content as an indicator of stress tolerance

Understanding the physiological reactions of *H. brevisubulatum* associations 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 effects that lead to decreased plant growth and production (Ashraf & Harris, 2003; Dubey, 2005; Hamilton et al., 2012). 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. It is suggested that the effect of the endophyte depended on the level of stress due to NaCl. These results were consistent with findings by Chen, Johnson, et al. (2018), who found *E. bromicola*-infected *H. brevisubulatum* plants also demonstrated improved photosynthesis under salt stress and alkali stress.

4.4 | Protection from oxidative stress

Increase in 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 & Nan, 2007) and active nodule colonization increases salt tolerance to alfalfa by increasing the activity of antioxidant enzymes (Wang, 2009).

The stability of cell membranes can be used to determine resistance of plants to oxidative stress, and the content of 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 *Medicago sativa* plants infected with fungus *Piriformospora 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 adjusts plants to high salt soils. Nagabhyru, Dinkins, Wood, Bacon, and Schardl (2013) reported *Festuca arundinacea* plants infected with fungus *Neotyphodium coenophialum* had higher levels of metabolites in shoots and roots under water-deficit stress.

4.5 | 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 *E. bromicola*

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. Endophyte infection has been found to increase germination in *Schedonorus arundinaceus* (Pinkerton, Rice, & Undersander, 1990), *Bromus setifolius* (Novas, Gentile, & Cabral, 2003), *Lolium multiflorum* (Gundel, Maseda, Vila-Aiub, Ghera, & Benech-Arnold, 2006), *F. rubra* (Wäli, Helander, Saloniemä, Ahlholm, & Saikkonen, 2005), *Poa leptocoma* (Kazenel et al., 2015) and *Lolium perenne* (Ma, Christensen, & Nan, 2015). The seed experiments indicate that the endophyte effects on germination of *H. brevisubulatum* seeds are 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. Similarly, endophyte infection has been found to increase germination under water-stress conditions (Zhang & Nan, 2010) and arsenic stress (Vazquez-de-Aldana et al., 2013). In contrast, *L. multiflorum* seeds infected with fungus *Neotyphodium* spp. (Gundel et al., 2006) and plants of *F. rubra* infected by *Epichloë festucae* (Gundel, Zabalgoceazcoa, & Vázquez de Aldana, 2011) reducing the percentage of germination under water potential pressure, but simultaneously increasing seed survival. These findings suggest that the endophyte plays important roles for seed germination under different conditions. In this research, *E. bromicola* endophyte enhanced germination under salt stress, and this may ensure more seedling establishment and enable *H. brevisubulatum* associations to withstand saline conditions.

5 | CONCLUSIONS

Results reported here demonstrate that the *E. bromicola* endophyte of 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 *E. bromicola* endophyte of wild barley appears to function in helping plants adapt 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 adapted for growth in alkalized meadows and other saline-alkali lands.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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