Interactive Effects of *Epichloë* Endophytes and Arbuscular Mycorrhizal Fungi on Saline-Alkali Stress Tolerance in Tall Fescue

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**Specialty section:** This article was submitted to Microbial Symbioses, a section of the journal Frontiers in Microbiology

**Received:** 16 January 2022  
**Accepted:** 10 March 2022  
**Published:** 25 April 2022

**Citation:** Liu H, Tang H, Ni X, Zhang Y and Wang Y (2022) Interactive Effects of *Epichloë* Endophytes and Arbuscular Mycorrhizal Fungi on Saline-Alkali Stress Tolerance in Tall Fescue. Front. Microbiol. 13:855890. doi: 10.3389/fmicb.2022.855890

**Keywords:** endophyte, arbuscular mycorrhizal fungi, interaction, tall fescue, saline-alkali stress

**INTRODUCTION**

*Epichloë* endophytes [Clavicipitaceae, Hypocreales, and Ascomycota] are endophytic fungi that can infect and coexist with above-ground portions of host plants and have been shown to impact their tolerance to certain biotic and abiotic stresses such as drought, low nitrogen, salt, cold, heat, heavy metals, insects, nematodes, and diseases by either enhancing the fitness and productivity of host plants or producing a range of alkaloids and other secondary metabolites (Schardl et al., 2004; Wang et al., 2021). In recent years, there has been a lot of research interest in the salt tolerance of hosts bearing *Epichloë* endophytes (Rodriguez et al., 2008; Reza and Mirlohi, 2010). Some studies found that under salt stress, endophyte infection enhanced growth, activity of enzymes of nitrogen metabolism, nitrogen use efficiency, and photosynthetic ability (Wang et al., 2021). In recent years, there has been a lot of research interest in the salt tolerance of hosts bearing *Epichloë* endophytes (Rodriguez et al., 2008; Reza and Mirlohi, 2010). Some studies found that under salt stress, endophyte infection enhanced growth, activity of enzymes of nitrogen metabolism, nitrogen use efficiency, and photosynthetic ability (Wang et al., 2019), and that *Hordeum brevisubulatum* infected with *Epichloë bromicola* had greater growth, including improved antioxidant potential, increased nutrient absorption, and osmotic and ionic adjustment (Song et al., 2015; Chen T. X. et al., 2018). Endophytes, on the other hand, may also decrease the salt tolerance of their hosts. For example, Ren et al. (2006) demonstrated that endophyte infection significantly decreased tiller number under high salt stress. Another study indicated that
endophytes did not influence the biomass production of *Festuca rubra* under soil salinity conditions (Zabalgozazcoa et al., 2006).

Plants can also deal with soil environmental stress by interacting with arbuscular mycorrhizal fungi (AMFs), which are members of the Glomeromycota phylum and form mutualistic relationships with more than 80% of terrestrial plant roots belowground and are found in saline and alkaline soils (Aliashgharzadeh et al., 2001; Wilde et al., 2009). Numerous studies have indicated that AMFs play an important role in improving host plant saline-alkaline stress tolerance by improving nutrient and water uptake, maintaining ion balance, increasing photosynthetic efficiency, and inducing the antioxidant defense system (Estrada et al., 2013; Abd-Allaa et al., 2019; Ben-Laouane et al., 2020; Moreira et al., 2020; Yang et al., 2020). In addition, Qiu et al. (2020) found that outcomes of salt stress tolerance of plants varied with AMF species identity. In addition, Parihar et al. (2020) indicated that multi-species AMF inoculation was superior to single AMF inoculation in improving plant production under salt stress conditions.

In grass species, dual infection of a host plant with leaf endophytes and below-ground AMFs is common. Interactions between grass endophytes and AMFs are complex, and studies focused on the two have been inconclusive based on relationships among the plant, AMFs, and grass endophytes. AMF colonization rates could be inhibited (Müller, 2003; Omacini et al., 2006; Mack and Rudgers, 2008) or enhanced (Novas et al., 2005, 2009; Arrieta et al., 2015) by leaf endophytes, and results are dependent on the combination of the AMFs and endophytes as well as soil environmental conditions. Our previous study has discovered that interactions between endophytes and AMFs, as well as their combined effects on growth of host plants depended on both the identity and richness of AMFs (Liu et al., 2019). In addition, Liu et al. (2011) demonstrated that the competition between AMFs and endophytes was determined by resource supply and host carbohydrate content. According to Zhou et al. (2016), tripartite interactions among endophytes, AMFs, and *Achnatherum sibiricum* were influenced by nutrient supply.

Many studies have been conducted to investigate the individual effects of *Epichloë* endophytes and AMFs on the growth of host plants and impact of saline-alkaline stress (Song et al., 2015; Chen T. X. et al., 2018; Wang et al., 2019; Moreira et al., 2020; Yang et al., 2020). Research on interactions of the two microorganisms infecting the same host plant is limited, especially regarding saline-alkali stress environments. This study was designed to evaluate the role of *Epichloë* endophytes and AMF species, as well as their interaction effects on the growth of tall fescue (*Lolium arundinaceum*) under saline-alkali stress conditions. As for AMFs, colonization with two AMFs, *Funneliformis mosseae* (FM) and *Claroideoglomus etunicatum* (CE), alone and in combination, as well as non-inoculated (M–), was considered. We analyzed the growth characteristics and physiological parameters of tall fescue. Specifically, we addressed the following questions: (1) does endophyte infection ameliorate saline-alkali stress performance of the host, and (2) when endophyte and AMF infections are both present, is the effect of endophyte infection on the host’s growth and saline-alkali tolerance influenced by the AMF?

### MATERIALS AND METHODS

**Plants and Fungi**

Endophyte-infected (E+) tall fescue seeds were naturally infected with *Epichloë coenophialum* (Morgan-Jones and Gams, 1982; Leuchtmann et al., 2014), and uninfected (E–) seeds were acquired by eliminating the endophyte by long-term storage of E+seeds at room temperature. This procedure reduces the viability of the endophyte but not the seeds (Clay and Holah, 1990). The seeds used in this experiment were several generations removed from the storage treatment and came from freely cross-pollinated field-grown parents. The E+ and E– seeds were provided by Professor Anzhi Ren at Nankai University. The E+ and E– seeds were sown in 160 pots (80 pots for E+ and 80 pots for E– plants), with 20 seeds per pot, in sterilized vermiculite. After germination, the seedlings were thinned to 15 per pot (21 cm in diameter × 16 cm in height). The endophyte status of the plants was checked both immediately before and after the experiment by microscopic examination of leaf sheaths stained with aniline blue, as described by Latch and Christensen (1985).

FM and CE were used in this study, and these two fungi were prepared by trap culture in pots using *Sorghum bicolor* as the host plant under controlled greenhouse conditions for 3 months. The soil was then dried, and the roots were cut into <1-cm pieces, which were subsequently homogenously mixed. The amount of inoculum for single species inoculation (FM or CE) was 100 g per pot; for the FM + CE treatment, the amount was 50 g for each AMF per pot. The M– treatment received a 100-g autoclaved inoculum and 50 ml of a non-autoclaved inoculum filtrate (passed through a 10-μm sieve) to correct for possible differences in the microbial community between the AMF and M– treatments.

**Experimental Design**

The experiment was conducted from October 15, 2020 to February 15, 2021 based on a fully crossed $2 \times 4 \times 4$ factorial design. The first factor, endophyte infection status, contained two levels: E+ and E–. The second factor, AMF inoculum, contained four levels: M–, inoculation with FM, inoculation with CE, and a combination inoculum of FM + CE. The third factor, saline-alkali treatment, contained four intensities: 0, 200, 400, and 600 mM. Four salts, NaCl, Na$_2$SO$_4$, NaHCO$_3$, and Na$_2$CO$_3$, were mixed in 9:1:1:9 M ratios to simulate a range of mixed saline-alkali stress conditions. There were 32 treatment combinations in total, and each was repeated five times. During the first 6 weeks, tall fescue seedlings were grown free of saline-alkali to ensure functional mycorrhiza and to avoid the effects of saline-alkali stress on fungi development (Feng et al., 2002). The seedlings were treated with modified 1/2 strength Hoagland nutrient solution supplemented with additional 0–, 200–, 400–, and 600–mM, as well as with 50 mM saline-alkali on the first and second days to avoid saline-alkali shock. Final concentrations of saline-alkali in each treatment were applied from the third day onward. Every 3 days, the treatment solution was replaced to maintain consistent stress conditions, and the position of each pot was changed randomly.
Harvest and Measurements
After 8 weeks of saline-alkali treatment, the shoots and roots were separated and harvested. Each pot’s roots were washed and divided into two sub-samples. AMF colonization was measured using a sample of approximately 2 g that was cleared in 10% KOH and stained in 1% trypan blue (Phillips and Hayman, 1970). AMF colonization rate was recorded using the cross-hair eyepiece method under a dissecting microscope at 40 × magnification (Mc Gonigle et al., 1990). The remainder of the sample was used to calculate biomass and other parameters. The biomass of the plants was determined after roots and shoots were oven dried at 80°C for 24 h. The plants were ground and digested at a high temperature in a PerkinElmer microwave using an HNO₃:HCl mixture (9:1). Phosphorus concentration was measured using acid-dissolved molybdenum, antimony, and scandium colorimetry (Chen et al., 2017). The concentrations of Na⁺ and K⁺ were assessed using ICP-OES in an Optima 7000 DV spectrophotometer (PerkinElmer, United States). Plant organic carbon (C) concentration in the shoots and roots was measured using the K₂CrO₇-H₂SO₄ oxidation method (Tanveer et al., 2014). Total nitrogen (N) was determined using a dry combustion method and an elemental analyzer (Vario EL/micro cube, Elementar, Hanau, Germany; Zhou et al., 2016).

Statistical Analyses
The effects of *Epichloë* endophytes, AMFs, and saline-alkali stress on AMF colonization rate, plant biomass, nutrient concentration parameters (C, N, and P), and cations (Na⁺ and K⁺) were analyzed by three-factor ANOVA with SPSS 20.0 (SPSS Inc., Chicago, IL, United States). When a significant effect was detected, differences in means between different treatments were determined by Duncan’s multiple range tests at a probability of 0.05. A redundancy analysis (RDA) was performed using *Epichloë* endophytes, AMF treatments, and saline-alkali stress (S) as explanatory variables, with growth (biomass) and physiological (C, N, P, Na⁺, and K⁺) parameters as response variables. After a Monte Carlo permutation test with 499 permutations, statistical significance was determined by stepwise forward selection.

RESULTS

Arbuscular Mycorrhizal Fungi Colonization Rate
AMF structures were not observed in the roots of M- treatments, whereas plants inoculated with AMF showed 9–63.3% root colonization rate. AMF colonization rate was significantly influenced by the *Epichloë* endophyte × saline-alkali stress interaction (Table 1). In the control treatment (0 mM), there was no difference in AMF colonization rate between E– and E+ plants, either with inoculation with FM or CE, or the mixture of FM and CE (FM + CE); however, higher colonization rate was found in E+ than in E– plants when inoculated with CE and the mixture of FM and CE (FM + CE) in the 200–, 400–, and 600-mM saline-alkali stress treatments, and no significant difference was observed between the E+ and E– plants when inoculated with FM (Figure 1).

Growth Parameters
The endophytes, AMFs, and saline-alkali stress all had a significant interactive effect on shoot and root biomass (Table 1). In the control treatment (0 mM), E+ had comparable shoot and root biomass to the E– plants regardless of AMF inoculation. Under saline-alkali conditions, with the M- treatment, the shoot and root biomass of the E+ plants was greater than that of the E– plants in the 200- and 400-mM saline-alkali stress treatments. When inoculated with AMF alone, FM had a detrimental effect on shoot and root biomass, while CE was found to be beneficial to host growth in the 200- and 400-mM stress treatments. The combination of FM and CE (FM + CE) had a similar effect on host growth as the CE. The growth advantage of E+ relative to the E– plants was reduced by FM but increased by CE and those simultaneously containing the FM and CE mixture (FM + CE) in the 200- and 400-mM saline-alkali treatments. No obvious difference was observed between the E+ and E– plants in the 600-mM saline-alkali treatment regardless of AMF inoculation (Figures 2A,B).

Carbon, Nitrogen, and Phosphorus Concentrations
C concentration in the shoots and roots showed no significant difference between the E+ and E– plants in the control treatment (0 mM). However, in the M- treatment, C concentration under stress (200, 400, and 600 mM) conditions was higher in E+ than in the E– plants in both the shoots and roots. FM inoculation significantly decreased C concentration in the shoots and roots of both the E+ and E– plants, and there was no difference between the E+ and E– plants when inoculated with FM either in the 200–, 400–, or 600-mM saline-alkali treatment. Unlike FM, the E+ plants had a higher C concentration in the shoots and roots than the E– plants when inoculated with CE, and a synergistic effect between CE and the endophytes was observed, with C concentration in the shoots and roots of host plants simultaneously infected by CE and endophytes being significantly greater than that of plants infected with either CE or the endophytes separately. The combination of FM and CE (FM + CE) had an effect on C concentration similar to that of CE (Table 2 and Figures 3A,B).

Saline-alkali stress significantly decreased the N and P concentration in the shoots and roots, and the decrease was significantly influenced by *Epichloë* endophyte × AMF (Table 2). In shoots and roots, the E+ plants had approximately 37 and 31% higher N concentrations, and 41 and 33% P concentrations, respectively, than the E– plants. The beneficial effects of endophytes were inhibited by the detrimental AMF, FM, and promoted by the beneficial AMF, CE, and the mixture of FM and CE (FM + CE) in the stress treatments (Figures 4 and 5).

Na⁺ and K⁺ Concentration
The results showed that as the stress concentrations increased, the Na⁺ concentration in the shoots and roots of tall fescue
### TABLE 1

Three-way ANOVA for the effects of endophytes (E), arbuscular mycorrhizal fungi (AMFs, M), and saline-alkali stress (S) on AMF colonization rate, biomass, Na\(^+\) and K\(^+\) in shoots and roots of tall fescue.

|                | AMF colonization rate | Biomass | Na\(^+\) | K\(^+\) |
|----------------|----------------------|---------|----------|---------|
|                | Shoot | Root | Shoot | Root | Shoot | Root |
| Endophytes (E) | 37.768*** | 30.789*** | 141.524*** | 77.542*** | 1667.281*** | 98.574*** | 37.151*** |
| AMF (M)        | 20.784*** | 109.944*** | 217.096*** | 126.138*** | 1201.875*** | 219.637*** | 58.935*** |
| Saline-alkali (S) | 9.097*** | 595.509*** | 1080.803*** | 2348.554*** | 1719.196*** | 1467.438*** | 232.668*** |
| E × M          | 10.995*** | 8.433*** | 11.416*** | 37.741*** | 205.126*** | 19.442*** | 3.913*** |
| E × S          | 5.149** | 11.877*** | 43.068*** | 9.015*** | 134.329*** | 13.780*** | 1.742ns |
| M × S          | 0.271ng | 12.315*** | 50.434*** | 21.773*** | 195.896*** | 23.651*** | 1.877ng |
| E × M × S      | 1.051ng | 2.677*  | 3.668**  | 6.323*** | 52.708*** | 1.293ng | 1.086ng |

The numeric data in the table is F-value. *, **, ***, and ns represent significance at the P < 5, 1, and 0.1% levels and non-significance, respectively.

**FIGURE 1** Arbuscular mycorrhizal fungi (AMF) colonization rate of tall fescue with (E+) and without (E–) endophytes and colonized with (AMF) and without (M-) AMF under saline-alkali stress. Values are means ± SE. Different letters denote means that are significantly different (P < 0.05).

**FIGURE 2** Biomass in shoots and roots of tall fescue with (E+) and without (E–) endophytes and colonized with (AMF) and without (M-) AMF under saline-alkali stress. (A) shoot biomass, (B) root biomass. Values are means ± SE. Different letters denote means that are significantly different (P < 0.05).
TABLE 2 | Three-way ANOVA for the effects of endophytes (E), AMFs (M), and saline-alkali stress (S) on C, N, and P concentrations in shoots and roots of tall fescue.

|           | C concentration | N concentration | P concentration |
|-----------|-----------------|-----------------|-----------------|
|           | Shoot            | Root            | Shoot           | Root           | Shoot            | Root            |
| Endophytes (E) | 203.822***      | 546.880***      | 290.541***      | 178.755***     | 818.466***      | 84.781***      |
| AMF (M)    | 362.740***      | 1458.130***     | 425.737***      | 565.720***     | 1780.575***     | 336.368***     |
| Saline-alkali (S) | 488.028***      | 536.472***      | 272.580***      | 283.243***     | 15.725***       | 73.061***      |
| E × M      | 41.748***       | 42.618***       | 16.169***       | 20.557***      | 111.896***      | 10.766***      |
| E × S      | 31.284***       | 62.494***       | 28.369***       | 28.032***      | 46.050***       | 2.383**ns      |
| M × S      | 24.002***       | 14.596***       | 18.059***       | 56.267***      | 142.904***      | 4.155***       |
| E × M × S  | 7.830***        | 7.876***        | 11.952***       | 5.721***       | 11.976***       | 2.306*         |

The numeric data in the table is F-value. *, **, *** and ns represent significance at the P < 0.5, 1, and 0.1% levels and non-significance, respectively.

The numeric data in the table is F-value. *, **, *** and ns represent significance at the P < 0.5, 1, and 0.1% levels and non-significance, respectively.

FIGURE 3 | Carbon (C) concentration in shoots and roots of tall fescue with (E+) and without (E–) endophytes and colonized with (AMF) and without (M-) AMF under saline-alkali stress. (A) shoot C concentration, (B) root C concentration. Values are means ± SE. Different letters denote means that are significantly different (P < 0.05).

FIGURE 4 | Nitrogen (N) concentration in shoots and roots of tall fescue with (E+) and without (E–) endophytes and colonized with (AMF) and without (M-) AMF under saline-alkali stress. (A) shoot N concentration, (B) root N concentration. Values are means ± SE. Different letters denote means that are significantly different (P < 0.05).

increased. However, *Epichloë* endophyte presence alleviated this change, with the E+ plants having significantly lower Na\(^+\) concentrations in the shoots and roots than the E– plants in the M- treatment. FM inoculation increased Na\(^+\) concentration when compared to the M- treatment, especially in the roots. CE inoculation decreased Na\(^+\) concentration when compared to the M- treatment. The mixture of FM and CE (FM + CE) had an effect similar to that of CE. In addition, there was a significant interaction between the endophytes and AMF species identity on Na\(^+\) concentration, with the E+ plants having higher shoot and root Na\(^+\) concentrations than the E– plants when inoculated with FM but lower shoot and root Na\(^+\) concentrations when
inoculated with CE and that containing both FM and CE mixture (FM + CE; Tables 1 and 3).

A decrease in K⁺ concentration was observed in the shoots and roots as the stress concentrations increased. However, the presence of the endophytes alleviated the decrease, particularly in shoot K⁺ concentration. In the M⁻ treatment, the E⁺ plants had significantly higher K⁺ concentrations in the shoots in the 200⁻, 400⁻, and 600-M M saline-alkali treatments, as well as a higher K⁺ concentration in the roots in the 200⁻ M saline-alkali treatments. FM inoculation had no significant effect on K⁺ concentration in shoots and roots of THE E⁺ plants, resulting in disappearance of the E⁺ advantage over the E⁻ plants. Under all stress conditions, a synergistic effect occurred between CE and the endophytes, and K⁺ concentration in the shoots and roots of host plants simultaneously infected by CE and the endophytes was significantly greater than that of plants infected with either CE or the endophytes separately (Tables 1 and 3).

**Redundancy Analysis**

Three factors, *Epichloë* endophytes, AMFs, and saline-alkali stress (S), as well as growth and physiological parameters of host plants, biomass, nutrients (C, N, and P), and cation (Na⁺ and K⁺) concentration, were used for RDA to investigate the contributions of both the endophytes and the AMFs to the growth of tall fescue under saline-alkali stress conditions. Axis 1 of the

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**TABLE 3** | Na⁺ and K⁺ concentrations in shoots and roots of tall fescue with (E⁺) and without (E⁻) endophytes and colonized with (AMF) and without (M⁻) AMF under saline-alkali stress (mean ± SE, n = 3).

|          | Na⁺          | K⁺          |
|----------|--------------|-------------|
|          | Shoot        | Root        | Shoot        | Root        |
|          | E⁻           | E⁺          | E⁻           | E⁺          |
| 0        | M⁻           | 0.19 ± 0.01m| 0.20 ± 0.01m| 0.22 ± 0.01r| 0.19 ± 0.01st| 3.37 ± 0.11ab| 3.39 ± 0.10ab| 1.73 ± 0.08cd| 1.79 ± 0.03bc|
|          | FM           | 0.16 ± 0.01m| 0.17 ± 0.01m| 0.18 ± 0.01st| 0.18 ± 0.01| 3.21 ± 0.01bcd| 3.11 ± 0.00de| 1.48 ± 0.05efgh| 1.57 ± 0.06defg|
|          | CE           | 0.22 ± 0.01m| 0.17 ± 0.00m| 0.20 ± 0.01rs| 0.15 ± 0.01u| 3.31 ± 0.02abc| 3.42 ± 0.07a| 2.09 ± 0.03a| 2.03 ± 0.04a|
|          | FM + CE      | 0.14 ± 0.02m| 0.15 ± 0.02m| 0.15 ± 0.01u| 0.15 ± 0.01u| 3.27 ± 0.05abcd| 3.16 ± 0.05cd| 1.98 ± 0.07ab| 2.09 ± 0.06a|
| 200 M⁻   | 0.95 ± 0.05j| 0.83 ± 0.01jk| 0.75 ± 0.01| 0.75 ± 0.03| 2.04 ± 0.05hij| 2.49 ± 0.02f| 3.11 ± 0.15hij| 1.57 ± 0.12defg|
|          | FM           | 1.08 ± 0.04hi| 1.18 ± 0.04gh| 0.82 ± 0.01hi| 0.85 ± 0.01g| 2.05 ± 0.01hij| 1.96 ± 0.07ij| 1.26 ± 0.06hijkl| 1.26 ± 0.05hijkl|
|          | CE           | 1.00 ± 0.07i| 0.63 ± 0.08i| 0.56 ± 0.00n| 0.40 ± 0.01q| 2.49 ± 0.09f| 2.95 ± 0.02e| 1.55 ± 0.07def| 1.81 ± 0.06bc|
|          | FM + CE      | 1.07 ± 0.04hi| 0.75 ± 0.02k| 0.62 ± 0.01i| 0.42 ± 0.00pq| 2.23 ± 0.06gh| 2.57 ± 0.04f| 1.42 ± 0.05gh| 1.65 ± 0.07cde|
| 400 M⁻   | 1.34 ± 0.06f| 0.99 ± 0.09i| 0.92 ± 0.01e| 0.62 ± 0.01l| 1.51 ± 0.03k| 1.89 ± 0.03j| 1.17 ± 0.10ijk| 1.31 ± 0.06hij|
|          | FM           | 1.26 ± 0.00g| 1.64 ± 0.05d| 0.83 ± 0.00g| 0.81 ± 0.00i| 1.34 ± 0.12km| 1.29 ± 0.06km| 1.11 ± 0.04jk| 1.07 ± 0.06kmn|
|          | CE           | 1.07 ± 0.03hi| 0.77 ± 0.03k| 0.60 ± 0.00m| 0.44 ± 0.00p| 2.15 ± 0.04n| 2.55 ± 0.04f| 1.37 ± 0.10hij| 1.63 ± 0.07cdf|
|          | FM + CE      | 1.14 ± 0.05gh| 0.85 ± 0.03jk| 0.70 ± 0.01k| 0.51 ± 0.000| 2.12 ± 0.06h| 2.39 ± 0.04fg| 1.14 ± 0.10jk| 1.42 ± 0.09gh|
| 600 M⁻   | 2.01 ± 0.02b| 1.75 ± 0.02cd| 1.19 ± 0.03a| 1.15 ± 0.01b| 1.10 ± 0.06n| 1.54 ± 0.07k| 0.89 ± 0.01op| 1.07 ± 0.09mno|
|          | FM           | 2.31 ± 0.03a| 2.36 ± 0.10a| 1.09 ± 0.01c| 1.10 ± 0.01c| 0.81 ± 0.03o| 0.82 ± 0.05o| 0.82 ± 0.01p| 0.79 ± 0.01p|
|          | CE           | 1.78 ± 0.02c| 1.46 ± 0.03e| 1.05 ± 0.01d| 0.84 ± 0.01gh| 1.52 ± 0.08kl| 1.95 ± 0.02ij| 0.99 ± 0.01mnop| 1.29 ± 0.01hijk|
|          | FM + CE      | 1.95 ± 0.05b| 1.68 ± 0.05cd| 1.17 ± 0.01b| 0.89 ± 0.01l| 1.48 ± 0.04km| 1.86 ± 0.09j| 0.92 ± 0.04nop| 1.16 ± 0.05ijklm|
inhibition of enzymes, disrupting K\(^+\) can alleviate the damage of plant cells by decreasing the Na\(^+\) concentration under saline-alkali stress, which was consistent with the findings of Song et al. (2015). The reduction of Na\(^+\) under saline-alkali conditions, which was consistent with previous research results (Chen T. X. et al., 2018; Wang et al., 2021). Third, the E+ plants had higher concentrations of C, N, and P in the shoots and roots than the E– plants under saline-alkali stress conditions, which can improve plant metabolism by promoting protein synthesis and increasing the concentration of compatible osmolytes, as well as helping to maintain cell membrane integrity and reducing electrolyte leakage (Song et al., 2015; Chen T. X. et al., 2018; Wang et al., 2021).

Both *Epichloë* and AMF metabolize carbohydrates from host plants. Previous studies have produced inconsistent results when *Epichloë* and AMF simultaneously interact with a host, reporting that AMF root colonization rate was inhibited (Omacini et al., 2006; Liu et al., 2011) or promoted (Novas et al., 2005; Vignale et al., 2016) by *Epichloë* endophyte infection. In our study, we demonstrated that *Epichloë* and saline-alkali stress had significant interactive effects on AMF colonization rate. Under the control treatment, *Epichloë* endophyte infection had no significant effects on AMF colonization rate regardless of AMF species. Under the saline-alkali stress treatment, the presence of *Epichloë* endophytes significantly decreased the colonization of FM but increased the colonization rate of CE and the mixture of FM and CE (FM + CE). These discrepancies of *Epichloë* endophytes in AMF colonization may be attributed to AMF species (Larimer et al., 2012; Zhou et al., 2016) and environmental conditions (Li et al., 2019). Larimer et al. (2012) showed that *Epichloë* elymi significantly promoted the colonization rate of FM but inhibited the colonization rate of *Claroideoglosum claroideum* (CC). Zhou et al. (2016) found that *Epichloë* endophyte infection significantly reduced the colonization rate of CE but did not affect the colonization rate of FM. Li et al. (2019) demonstrated that the effect of *Epichloë* endophyte infection on the colonization rate of CE was influenced by soil water content. Endophyte infection significantly reduced the colonization rate of CE at 70% soil water content but had no effect on that of CE at either 50 or 30% soil water content.

When tripartite interactions among *Epichloë* endophytes, AMFs, and host plants were considered, some studies found that the effects of *Epichloë* endophyte infection on host plants were influenced by AMFs (Zhou et al., 2016; Liu et al., 2017, 2019; Li et al., 2019). For example, Zhou et al. (2016) reported that the effects of *Epichloë* endophyte infection on the shoot biomass of *A. sibiricum* changed from neutral with the M- treatment to positive with the FM inoculation treatment under sufficient N and P conditions. Li et al. (2019) indicated that there was no significant effect of *Epichloë* endophyte infection on the total P content of *Lolium perenne* with the M- treatment but significantly increased total P content with AMF inoculation treatment under 70% soil water content conditions. Liu et al. (2019) discovered that the outcomes of tripartite interactions among *Epichloë* endophytes, AMFs, and host plants varied with AMF identity. The beneficial effect of endophyte infection

RDA plot explained 71.4% of the total variance by being positively correlated with endophytes and CE and negatively correlated with S and FM. Axis 2 explained 13.1% of the total variance. The endophytes, FM, CE, and S explained 4, 15, 36, and 44% of the total variance, respectively (Figure 6).

**DISCUSSION**

Many studies have reported the *Epichloë* endophytes’ ability to increase host resistance to abiotic stresses (Wang J. F. et al., 2020; Wang Z. F. et al., 2020; Chen et al., 2021; Liu et al., 2021; Wang et al., 2021). Among abiotic stress conditions, we demonstrated that *Epichloë*-infected tall fescue outperformed uninfected plants in saline-alkali soil. The ability of *Epichloë* to improve resistance to NaCl stress, a single physiological stress, has been demonstrated in many studies (Chen S. et al., 2019; Chen T. X. et al., 2018, 2019; Wang et al., 2019; Chen et al., 2021); however, very little is known about how *Epichloë* infection impacts mixed saline-alkali stress. The results of our study, first, revealed that endophyte presence resulted in higher biomass of shoots and roots under saline-alkali stress (200 and 400 mM) relative to that of the E– plants, which was consistent with previous research results (Chen T. X. et al., 2018, 2019; Wang et al., 2019). Second, *Epichloë* endophyte infection significantly decreased the concentration of Na\(^+\) and increased the concentration of K\(^+\) under saline-alkali conditions, which was consistent with the findings of Song et al. (2015). The reduction of Na\(^+\) due to endophytes can alleviate the damage of plant cells by decreasing the inhibition of enzymes, disrupting K\(^+\) acquisition, and inhibiting K\(^+\)-dependent metabolic processes on the one hand, and reducing oxidative stress on the other (Chen T. X. et al., 2018; Chen T. X. et al., 2019; Chen J. B. et al., 2019). Under saline-alkali stress, K\(^+\) accumulation by endophytes is important for stomatal conductance and maintenance of normal plant activities (Chen J. B. et al., 2019). *Epichloë* endophytes regulate the balance of Na\(^+\) and K\(^+\) in plants, thus maintaining normal metabolic processes in cells and improving the adaptation of plants to saline-alkali environments (Song et al., 2015; Chen S. et al., 2018; Wang et al., 2021).
on plant shoot biomass decreased in response to FM but increased in response to *Rhizophagus intraradices* (RI). No obvious difference was observed between the E+ and E–-plants when inoculated either with CE or CC. Some studies, however, reported no interaction between *Epichloë* endophytes and AMFs in host plants (Omacini et al., 2006; Mack and Rudgers, 2008; Larimer et al., 2012). In our study, we found that the interaction between *Epichloë* endophytes and AMFs has a significant influence on the saline-alkali resistance of tall fescue, and that this was dependent on the species of AMFs. For the E– plants, host plant resistance to saline-alkali stress was decreased by FM but increased by CE. FM inoculation significantly decreased plant biomass (shoot and root biomass), nutrient concentrations (C, N, and P), and K⁺ concentration while increasing Na⁺ concentration. Contrary to FM, CE inoculation significantly increased the differences between the E+ and E– plants under saline-alkali stress conditions were reduced by FM but increased by CE.

According to the RDA analysis, the contribution of endophytes was less than that of AMFs and further suggested that the AMF species played an important role in *Epichloë* endophyte-host-AMF tripartite interactions. This result was similar to the findings of Zhou et al. (2016) who found that the contribution of endophytes to *A. sibiricum* was less than that of AMFs under conditions of nutrient stress. AMFs were present in the roots of host plants, and they directly absorbed N and P via external AMF hyphae in soil (Kong et al., 2019), whereas *Epichloë* endophytes lived in the above-ground tissues of the host plants (Wang J. F. et al., 2020) and indirectly affected nutrient absorption by changing the root’s morphological and physiological characteristics (Chen et al., 2020, 2021). This may be the reason that could explain why *Epichloë* endophytes have lower contribution than AMFs under saline-alkali stress conditions.

**CONCLUSION**

In conclusion, a significant interaction among the *Epichloë* endophytes, AMFs, and saline-alkali stress occurred in tall fescue growth and physiological parameters. Endophyte infection significantly enhanced tall fescue resistance to saline-alkali stress by increasing biomass, nutrient uptake, and accumulation of K⁺ while decreasing Na⁺ concentration; this beneficial effect of the endophytes was enhanced by the beneficial AMF, CE, but reduced by the detrimental AMF, FM. Our study reinforces the currently limited finding that *Epichloë* endophytes and AMFs interact in complex ways to influence the growth of their shared host grasses, especially under stress conditions. Further research should be conducted to investigate the ecological implications of the combined effects of *Epichloë* endophytes and AMFs under field conditions.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in this study are included in the article supplementary material, further inquiries can be directed to the corresponding author.

**AUTHOR CONTRIBUTIONS**

HL designed the research and revised and polished the manuscript. HT, XN, YZ, and YW performed the experiments. HL and HT analyzed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

**FUNDING**

This study was supported by the National Natural Science Foundation of China (32001103) and Dezhou University Science Research Foundation (2019jrc317).

**REFERENCES**

Abd-Allaa, M. H., Nafadya, N. A., Bashandya, S. R., and Hassan, A. A. (2019). Mitigation of salt stress on the nodulation, nitrogen fixation and growth of chickpea (*Cicer arrietinum* L.) by triple microbial inoculation. *Rhizosphere* 10:100148. doi: 10.1016/j.rhisp.2019.100148

Aliasgharzadeh, N., Rastin, S. N., Towfighi, H., and Alizadeh, A. (2001). Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. *Mycorhiza* 11, 119–122. doi: 10.1007/s005720100113

Arrieta, A., Iannones, L. J., Scervino, J., Vignale, M., and Novas, M. (2015). A foliar endophyte increases the diversity of phosphorus-solubilizing rhizospheric fungi and mycorrhizal colonization in the wild grass *Bromus auleticus*. *Fungal Ecol.* 17, 146–154. doi: 10.1016/j.funeco.2015.07.001

Ben-Laouane, R., Baslam, M., Ait-El-Mokhtar, M., Anli, M., Boutasknit, A., Ait-Rahou, Y., et al. (2020). Potential of native arbuscular mycorrhizal fungi, rhizobia, and/or green compost as *Allafia* (*Medicago sativa*) enhancers under salinity. *Microorganisms* 8:1695. doi: 10.3390/microorganisms81 11695

Chen, J. B., Zong, J. Q., Li, D. D., Chen, Y., Wang, Y., Guo, H. L., et al. (2019). Growth response and ion homeostasis in two bermudagrass (*Cynodon dactylon*) cultivars differing in salinity tolerance under salinity stress. *Soil Sci. Plant Nutr.* 4, 419–429. doi: 10.1080/00380768.2019.1631125

Chen, T. X., Li, C. J., White, J. F., and Nan, Z. B. (2019). Effect of the fungal endophyte *Epichloë bromicola* on polyamines in wild barley (*Hordeum brevisulcubatum*) under salt stress. *Plant Soil* 436, 29–48. doi: 10.1007/s11104-018-03913-x

Chen, S., Chen, T., Yao, X., and Lv, H. U. I. (2018). Physicochemical properties of an asexual *Epichloë* endophyte modified wild barely in the presence of salt stress. *Pak. J. Bot.* 50, 2105–2111.

Chen, T. X., Johnson, R., Chen, S. H., Lv, H., Zhou, J., and Li, C. J. (2018). Infection by the fungal endophyte *Epichloë bromicola* enhances the tolerance of wild barley (*Hordeum brevisulcubatum*) to salt and alkali stresses. *Plant Soil* 428, 353–370. doi: 10.1007/s11104-018-3643-4

Chen, T. X., White, J. F., and Li, C. J. (2021). Fungal endophyte *Epichloë bromicola* infection regulates anatomical changes to account for salt stress tolerance in wild barley (*Hordeum brevisulcubatum*). *Plant Soil* 461, 533–546. doi: 10.1007/s11104-021-08428-w
Chen, Y. L., Deng, Y., Ding, J. Z., Hu, H. W., Xu, T. L., Li, F., et al. (2017). Distinct microbial communities in the active and permafrost layers on the Tibetan Plateau. Mol. Ecol. 26, 6608–6620. doi: 10.1111/mec.13496

Chen, Z. J., Jin, Y. Y., Yao, X., Chen, T. X., Wei, X. K., Li, C. J., et al. (2020). Fungal endophyte improves survival of Lolium perenne in low fertility soils by increasing root growth, metabolic activity and absorption of nutrients. Plant Soil 452, 185–206. doi:10.1007/s11104-020-04656-7

Clay, K., and Holah, J. (1990). Fungal endophyte symbiosis and plant diversity in successional fields. Science 285, 1742–1745. doi:10.1126/science.285.5434.1742

Estrada, B., Aroca, R., Maathuis, F. J. M., Barea, J. M., and Ruiz-Lozano, J. M. (2013). Arbuscular mycorrhizal fungi native from a Mediterranean saline area enhance maize tolerance to salinity through improved ion homeostasis. Plant Cell Environ. 36, 1771–1782. doi:10.1111/jpe.12082

Leuchtmann, A., Bacon, C. W., Schardl, C. L., White, J. F., and Tadych, M. (2014). Nomenclatural realignment of Neotyphodium spp. fungi in Lolium perenne. Mycotaxon 131, 35–46. doi:10.5248/131.01

Latch, G. C. M., and Christensen, M. J. (1985). Artificial infection of grasses by vesicular-arbuscular mycorrhizal fungi. Mycologia 77, 791–799. doi:10.1080/00275514.1990.10645051

Li, H., Courchesne, S., and Beare, E. (2018). Effect of Melilotus albus infection on the root growth of Lolium perenne. Funct. Plant Biol. 45, 757–766. doi:10.1071/FP17200

Liu, Y. L., Hou, W. P., Jin, J., Christensen, M. J., Gu, L. J., Cheng, C., et al. (2021). Epichloë gansuensis increases the tolerance of Achnatherum inebratais to low-P stress by modulating amino acids metabolism and phosphorus utilization efficiency. J. Fungi 7:390. doi:10.3390/jof7050390

Mack, K. M. L., and Rudgers, J. A. (2008). Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. Oikos 117, 310–320. doi:10.1111/j.0030-1299.15973.x

McConigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., and Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115, 495–501. doi:10.1111/j.1469-8137.1990.tb00476.x

Moreira, H., Pereira, S. I. A., Vega, A., Castro, P. M. L., and Marques, A. P. G. C. (2020). Synergistic effects of arbuscular mycorrhizal fungi and plant growth-promoting bacteria benefit maize growth under increasing soil salinity. J. Environ. Manage. 257:109982. doi:10.1016/j.jenvman.2019.109982

Phillips, J. M., and Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–161. doi:10.1016/0075-1072(70)90110-3

Qiu, Y. J., Zhang, N. L., Zhang, L. L., Zhang, X. L., Wu, A. P., Huang, J. Y., et al. (2020). Mediation of arbuscular mycorrhizal fungi on growth and biochemical parameters of Ligustrum vicaryi in response to salinity. Physiol. Mol. Plant Pathol. 112:101522. doi:10.1016/j.pmpp.2020.101522

Ren, A. Z., Gao, Y. B., Zhang, J., and Zhang, J. (2006). Effect of endophyte infection on salt resistance of ryegrass. Acta Ecol. Sin. 26, 1750–1757.

Wang, J. F., Hou, W. P., Christensen, M. J., Zhang, J., and Zhang, J. (2006). Effect of endophyte infection on salt resistance in tall and meadow fescues. J. Plant Nutr. Soil Sci. 173, 952–957. doi:10.1002/pln.200900345

Yang, Y. R., Cao, Y. P., Li, Z. X., Zhukova, A., Yang, S. T., Wang, J. L., et al. (2014). Effect of root corn mulch and N fertilizer application on nitrous oxide (N₂O) emission and wheat crop productivity under rain-fed condition of Loess Plateau China. Int. J. Agric. Biol. 16, 505–512.

Vignale, M. V., Iannone, L. J., PinCET, A. D., De Battista, J. P., and Novas, M. V. (2016). Effect of epichloë endophytes and soil fertilization onarbuscular mycorrhizal colonization of a wild grass. Plant Soil 405, 279–287. doi:10.1007/s11104-015-2522-5

Wang, J. F., Hou, W. P., Christensen, M. J., Li, X. Z., Xia, C., Li, C. J., et al. (2020). Role of Epichloë endophytes in improving host grass resistance ability and soil properties. J. Agric. Food Chem. 68, 6944–6955. doi:10.1021/acs.jafc.0c01396

Wang, Z. F., Li, C. I., and White, J. (2020). Effects of Epichloë endophyte infection on growth, physiological properties and seed germination of wild barley under saline conditions. J. Agro. Crop Sci. 206, 43–51. doi:10.1111/jasc.12366

Wang, J. F., Hou, W. P., Christensen, M. J., Xia, C., Chen, T., Zhang, Z. X., et al. (2021). The fungal endophyte Epichloë gansuensis increases NaCl tolerance in Achnatherum inebratais through enhancing the activity of plasma membrane H⁺-ATPase and glucose-6-phosphate dehydrogenase. Sci. China Life Sci. 63, 452–465. doi:10.1007/s11427-020-1674-y

Wang, J. F., Tian, P., Christensen, M. J., Zhang, X. X., Li, C. J., and Nan, Z. B. (2019). Effect of Epichloë gansuensis endophyte on the activity of enzymes of nitrogen metabolism, nitrogen use efficiency and photosynthetic ability of Achnatherum inebratais under various NaCl concentrations. Plant Soil 435, 57–68. doi:10.1007/s11104-018-3868-2

Wilde, P., Manal, A., Stodden, M., Sieverding, E., Hildebrandt, U., and Bothe, H. (2009). Biodiversity of arbuscular mycorrhizal fungi in roots and soils of two salt marshes. Environ. Microbiol. 11, 1548–1561. doi:10.1111/j.1462-2920.2009.01882.x

Yang, Y. R., Cao, Y. P., Li, Z. X., Zhukova, A., Yang, S. T., Wang, J. L., et al. (2020). Interactive effects of exogenous melatonin and Rhizopus intraradices on saline-alkaline stress tolerance in Leymus chinensis. Mycorriza 30, 357–371. doi:10.1007/s00572-020-00942-2
Zabalgogeazcoa, I., Romo, M., Keck, E., Vázquez de Aldana, B. R., García Ciudad, A., and García Criado, B. (2006). The infection of Festuca rubra subsp. pruinosa by Epichloë festucae. Grass Forage Sci. 61, 71–76. doi: 10.1111/j.1365-2494.2006.00509.x

Zhou, Y., Li, X., Qin, J. H., Liu, H., Chen, W., Niu, Y., et al. (2016). Effects of simultaneous infections of endophytic fungi and arbuscular mycorrhizal fungi on the growth of their shared hostgrass Achnatherum sibiricum under varying N and P supply. Fungal Ecol. 20, 56–65. doi: 10.1016/j.funeco.2015.11.004

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