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Segregation of *Lolium perenne* into a subpopulation with high infection by endophyte *Epichloë festucae* var. *lolii* results in improved agronomic performance

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Abstract

Background and aims Low temperature stress is a common hazard during plant growth. Endophyte infection has been shown to increase cold tolerance in host plants. Many *Lolium perenne* cultivars contain low to moderate levels of endophyte. This study was done to explore cultivar improvement by segregation of endophyte containing individuals from the original cultivar to create a high endophyte subpopulation.

Methods Endophyte-infected plants were segregated over the first three years to produce high-endophyte subpopulation, and field and greenhouse experiments were carried out in the forth and fifth to determine the cold tolerance of the *L. perenne* subpopulation with high endophyte infection rates (N), the parent (F), the control endophyte-free subpopulation (E) and the control local variety (L).

Results (1) After three years of screening, high endophyte infection rates in the tillers and seeds of plants were still observed (96.5%), and agronomic traits (crown width, plant height, panicle number, withering, regreen-up, the growth cycle and the over-wintering rate) was also improved with increased *Epichloë* colonization of host plant. (2) The subpopulation with high endophyte infection rates and improved agronomic traits had better cold tolerance than the parent, the control endophyte-free subpopulation and the control local variety. The possible mechanisms by which high endophyte infection enhances cold resistance in the field include increased root system, increased the over-wintering rate, reduced regrowth periods with the sowing date being October 15th. (3) The high-endophyte subpopulation significantly increased SOD, POD, CAT, and APX activities at 0, 5, and 10 °C by 11.8%–44.6%, compared with the parent population.

Conclusions The subpopulation had a high endophyte infection rate, improved agronomic traits and higher enzymatic activities. These results indicate that increasing endophyte infection rates by selection, effectively improved agronomic traits and cold tolerance.

Keywords

Lolium perenne, *E. festucae* var. *lolii*, Endophyte infection rate, Cold tolerance, Sowing date, Low temperature stress

Introduction

Low temperature stress (LTS) can seriously damage and limit plant growth, which subsequently impacts on the development of sustainable agricultural practices in regions that are temperate or are at high elevations (Megha et al., 2017). Long-term LTS causes injuries to plants induced by both chilling (<20 °C) and freezing (<0 °C) (Zheng et al., 2016), that directly hamper key metabolic processes (Lee et al., 2002; Viswanathan et al., 2007). For example, LTS was the causal agent of nonfunctional chloroplasts, which limited the assimilation of CO_2 (Bilska and Sowiński, 2010) and, ultimately, caused cell death (Gómez et al., 2004). In addition, the excessive accumulation of reactive oxygen species (ROS) caused by LTS can result in detrimental changes to the cell membrane structure (Li et al., 2012; Scebba et al., 1998). Further, LTS can negatively impact root activity and development, resulting in the reduced uptake of nutrients and water (Hund, 2010; Hund et al., 2008). Overall, LTS can adversely affect the entire growth process of plants, from poor germination rates, plant growth retardation, reduced tillering, reduced fruit set, reduced yield, and reduced quality (Jha et al., 2017; Shimono et al., 2007).

Plants have developed a number of unique mechanisms to cope with LTS during the course of their evolution (Liu et al., 2015; Molinier et al., 2006). For instance, in temperate regions plants can acquire a certain degree of tolerance to very low temperatures after exposure to low but non-freezing temperatures, an adaptive process known as cold acclimation (Barrero-Gil et al., 2016; Kovi et al., 2015). Plants can also adapt to the cold by promoting the activity of ROS scavenging enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) activity (Chen et al., 2014), and by increasing the contents of non-enzymatic antioxidants, such as glutathione (GSH) and ascorbate (AsA) (Huang et al., 2018), which enhance cold tolerance. Accumulation of some metabolites (e.g., sugars, amino acids, polyamines, abscisic acid, and alkaloids) can also improve plant cold tolerance (Bhowmik et al., 2006; Faltusová-Kadlecová et al., 2002; Shen et al, 2000; Zhou et al., 2015). Additionally, some crop management practices can improve plant cold tolerance remarkably (Munshaw et al., 2010; Han and Han, 2015). For example, appropriate sowing dates are beneficial for plants that need to overwinter (Zhang et al., 2012). Numerous studies have shown that when plants are inoculated with microorganisms, cold tolerance is conferred (Redman et al.,

2011; Rodriguez and Redman, 2007; Yarzábal, 2014; Zhang et al., 2013). For example, Chen et al. (2016) found that the cold tolerance of *Achnatherum inebrians* plants inoculated with *Epichloë gansuensis* was significantly higher than that of uninoculated plants.

Epichloë endophytes are endosymbiotic microbes that live asymptomatically within plant tissues (Saikkonen et al., 1998). *Epichloë* endophytes have developed a symbiotic relationship with cool-season grasses (subfamily Poöideae) (Florea et al., 2015). The *Epichloë* symbionts are often vertically transmitted to the next generation via maternal seeds (Gundel et al., 2017). These endophytes are mainly distributed in the nutrient-rich parts of the grass, except for the roots (Christensen et al., 2008; Rasmussen et al., 2009). The host plants of *Epichloë* endophytes are conferred tolerance to biotic and abiotic forms of stress, including drought, cold, heat, salt, heavy metals, insects, and pathogens (Schardl et al., 2004; White et al., 2001), by producing a series of alkaloids and other secondary metabolites (Chen et al., 2018a). Some studies have shown that *Epichloë* endophytes play a major role in the cold tolerance of host plants (Chen et al., 2016; Zhou et al., 2015). However, the *Epichloë* endophyte removal did not reduce the cold tolerance of tall fescue (Casler and Santen, 2008). There may be differences in cold tolerance owing to endophytic fungal and host grass genetic diversity (Kover et al., 1997).

Perennial ryegrass is one of the most common forage and turfgrass species that is widely adaptable to a wide scope of adverse environment conditions. Based on the season, perennial ryegrass is often selected as a model species for studies on cold stress (Dalmannsdottir et al., 2017). Although *L. perenne* exhibits superior cold hardiness, winter injury is observed in northern China, especially, in the Qinghai-Tibet Plateau, where the winter is severe and prolonged (Zhang et al., 2000). Phylogenetic analyses indicate that *Epichloë festucae* var. *lolii* and ryegrass are symbionts (Leuchtmann et al., 2014). Some studies indicated that the infection rate by endophytes shows diversity (Clay and Schardl, 2002). This could be due to mycelial inactivation in seeds resulting from differential compatibility between plants and fungi. Because of the spatial distribution of host plants and seed transmission, the infection rates will also change accordingly in different locations, and over time, due to selection pressure of the environment (Malinowski and Belesky, 2010; Patchett et al., 2011). The trend of infection rate of the endophyte depends on the environmental conditions (Clay and Schardl, 2002). In the present study, using a microscopic examination technique, we found a different endophyte infection rate ranged from 0% to 100% in the infected plant tillers of the perennial ryegrass (Chen et al.,

2018b). Therefore, the primary aim of our study was to segregate a subpopulation of perennial ryegrass, in which endophyte infection rate of the tillers and seeds were consistently above 95%.

Taking into account the data from previous studies and reports, we hypothesized that: (1) high infection rates of grasses with endophytes would significantly improve their agronomic traits; (2) the subpopulation with high infection rates of endophyte (N) would possess improved cold tolerance than the parent (F), the control endophyte-free subpopulation (E) and the control local variety (L); and (3) the high endophyte subpopulation would show increased antioxidant enzyme activities, compared to those of the parent population. Therefore, field selection was carried out for three years to produce the high endophyte subpopulation. Then, the high endophyte subpopulation, the parent, the control endophyte-free subpopulation and the control local variety were sown at different sowing dates in the fields for over-wintering. Additionally, the *L. perenne* subpopulations with high-endophyte and the parent population were tested for cold tolerance under controlled conditions. The main aims of this study were to determine the effects of high infection rate of endophytes on important agronomic traits (crown width, plant height, panicle number, green stage, over-wintering rate, etc.), define the cold tolerance of the high-endophyte subpopulation, and identify the possible mechanisms of cold tolerance of the host subpopulations with the high infection rates of the endophytes.

Materials and methods

Experimental site

Field screening was conducted over four growing seasons, from 2014 to 2017, at the Yuzhong Experimental Station of Lanzhou University, Gansu Province, China (35°85′ N, 104°12′ E), at an elevation of 1400 m. The region has a temperate continental climate with an annual average temperature and precipitation of 8.1 °C and 342 mm during four experimental periods, respectively. The highest (July) and lowest (January) monthly averages for temperature during the screening period were 29.8 °C and -17.5 °C, respectively. Generally, LTS occurs from late October through March. Meteorological data were obtained from a weather station installed inside the experimental station.

Production of the high-endophyte subpopulation

Seed of the parent variety pinnacle was purchased from Barenbrug Seeds, China, and the average infection rate of the seeds

was 62.5%, determined by microscopic examination of aniline blue stained seeds (Song et al., 2015). Sowing was performed manually in early September in 2014, using a 50-cm row spacing and distance between plants within rows. Water was mainly supplied by sprinkler irrigation after planting. During the seedling period, manual weeding was done. Before harvesting, plant height, panicle number per plant, crown width, withering stage, green stage, green-up stage, and over-wintering rate were recorded.

Seeds per plant were harvested at maturity in July, and the infection rate of individual plants was determined by microscopic examination of stained tillers and seeds (Chen et al., 2017). Plants with high (≥95%) and low (≤2%) infection rates in the tillers and seeds were designated endophyte-infected (E+) and endophyte-free (E-), respectively. The plants were all screened for infection rates and agronomic traits. Seeds from the E+ and E− subpopulations were stored at 4 °C to break seed dormancy. Screening was repeated from within the E+ and E- subpopulations in 2015, and again from within the next generation of E+ and E- populations in 2016 using the same sowing and selection method as above. E+ and E- seeds were sown separately. The selection of subpopulations was completed after three years.

Field experiment (sowing rate treatment)

Induction of plant winter hardiness depends on the growth stage at the onset of winter (Urbatzka et al., 2012). Therefore, the high levels of endophyte subpopulation (N, 96.5%), the parent (F, 62.5%), the control endophyte-free subpopulation (E, 0%) and the control local variety (L, 4.2%) were planted separately in 2017 in a common field at the Yuzhong Experimental Station on different sowing dates (August 25th, September 19th, and October 15th) to evaluate cold tolerance. The experiment was laid in a randomized complete block design with five replicates; 240 g of seed was planted in each experimental plot (4 × 5 m) using a small lawn hand-sowing machine and covered with non-woven fabric. Sprinkle irrigation for 2 hours was used every morning and evening before seedling emergence to provide sufficient water availability for fast growth. Root length and diameter, depth and diameter of root neck, and withering stage before winter were measured and recorded. After over-wintering, the regreening stage and over-wintering rates were observed in 2018.

Field-data acquisition

Plant height, root length, and crown width were measured with a ruler. Root-neck depth was defined as the distance from the

upper part of the root neck to the soil surface and was measured using a steel rod. Root neck diameter (the coarsest width of the root neck) was measured with a vernier caliper (Mitutoyo Co., Kawasaki, Japan). Panicle number, speed of the turf formation, ground coverage, withering stage, beginning of green-up, and green stage, were measured visually (base on the withering and regreening of 50% plants).

Low temperature experiment

E+ and E- seeds from the subpopulations and the parent population were sown in four germination tray filled with vermiculite that was previously sterilized by incubation at 150 °C for 24 h, respectively. Modified half-strength Hoagland's nutrient solution (Ma et al., 2012) and water from irrigation were provided as required for all trays regularly at 7d intervals. After 15 days, following reconfirmation of the endophyte-infection status, seedlings were transplanted into 168 pots (21 cm diameter × 16 cm high; 5 seedlings per pot), 84 pots for E+ (F and N), and 84 pots for E- (F and N) filled with a mixture of sterilized vermiculite and Yuzhong loess (1:2). All trays were supplied with the same volume of nutrient solution. Plants were grown from June 6 to July 20, 2018, in a greenhouse at the College of Pastoral Agriculture Science and Technology, Lanzhou University, under long day conditions (16/8 light/dark, 25/16 °C day/night) and a light intensity of 800 μmol m^{-2} s^{-1}.

After 45 days, all pots were transferred into six LRH-250-G artificial-illumination incubators (Medical Equipment Factory, Hei Longjiang, China). Twenty-eight pots, 14 E+ pots (7 with F and 7 with N), and 14 E- pots (7 with F and 7 with N) were placed at random in each incubator at 0, 5, 10, 15, 20, and 25 °C for 25 days; the position of each pot was changed randomly at 3 d intervals in each incubator. For each pot, 600 ml nutrient solution and water were supplied regularly at 5 d intervals, to ensure normal plant growth and development.

Determination of enzymatic activities in low temperature

Upon termination of the temperature treatments, all of the aboveground parts were cut 2 cm above the soil, and residual aboveground parts and all roots were carefully removed from the growth medium in pots, and thoroughly washed with distilled water before manual separation into the root and stem parts. To determine enzymatic activity, stems, root hairs (separated from the root), and roots from the remainder of each treatment were separately collected and immediately frozen in liquid nitrogen and stored at −80 °C until use.

Enzymes were extracted from 0.5 g samples of stems, roots, and root hairs in extraction phosphate buffer (PBS, pH 7.8). Homogenates were centrifuged at 7500 × *g* for 8 min at 4 °C, and supernatants used to determine enzyme activity. SOD activity was measured by the method of Vestena et al. (2011) at 560 nm using a VIS-SP-723-type spectrophotometer (Spectrum Instruments, Co. Ltd., Shanghai, China); POD activity was measured by the guaiacol method as previously described (Garcíalara et al., 2007). CAT activity was assayed as reported by Lee et al. (2003). APX activity was determined at 290 nm with a UV-6100 double beam spectrophotometer (İşeri et al., 2013; Mohammadian et al., 2012).

Statistical analysis

Data analyses were performed with SPSS version 17.0 (SPSS, Inc., Chicago, IL). Two-way AVOVA was used to determine the effects of variety (V) and sowing date (D) on plant height, root length, root neck depth and diameter, and overwintering rate, as well as the effects of endophyte status (E_1), variety (V), stress level (S) and tissue (T) on SOD, POD, CAT and APX activities via multitude-comparison AVOVA. Significant differences between the high endophyte subpopulation and the other populations tested under the same treatment (sowing date or low temperature) were determined by Tukey's-b (k) test. Statistical significance was defined at the 95% confidence level.

Results

The infection rate of endophytes and the agronomic traits of the subpopulations

There were 128, 1860, and 16620 individual plants of E+ and E- harvested in 2015, 2016, and 2017; the average tiller number of E+ per plant was 68, 113, and 206, respectively, and were 1, 15, and 29 higher than that E- per plant. The average infection rate of E+ per plant observed by microscopic examination of the stained stalk-piths and seeds were 59.4%, 87.1%, and 93.6% for the tillers, and 65.7%, 89.1%, and 96.5% for the seeds in 2015, 2016, and 2017, respectively. The average infection rate of E- per plant in tillers and seeds decreased with the continuous screening (Table 1).

Table 1 Endophyte infection rate of E+ and E- plants in stems and seeds per plant during 2015-2017

Year	Detected per plants	Average tiller number per plant	Average infection rate of tiller per plant (%)	Average infection rate of seeds per plant (%)

	E+	E-	E+	E-	E+	E-	E+	E-
2015		128	68	67	59.4	5.6	65.7	6.0
2016	1000	860	113	98	87.1	1.6	89.1	2.2
2017	8960	7660	206	177	93.6	0.9	96.5	1.1

Plants with the high endophyte levels were confirmed to have infection rates above 96.5%, and were further screened for agronomic traits. The average crown width of E+ and E- per plants mainly focused on 8cm in 2015, while the average crown width in E+ and E- per plants changed with delays for screening time. In 2017, the average crown width of E+ per plants was 48.3 cm, and of E- per plants was 32cm (Table 2). The average height in E+ per plant was 32.4, 36.8, and 42.6 cm, and the number of E+ individual plants ranged from 30 to 45 cm of the average height accounted for 32.9%, 54.1%, and 70.3% of the measured plants in 2015, 2016, and 2017, respectively; while the average height of E- plants ranged from 15 to 30cm each year (Table 2). The average panicle number per plant of E+ plants was 82.5, 122.6, and 186.6 each year and ranged from above 80, respectively, and the average panicle number per plant of E- plants was 76.5, 119.2, and 178.8, and the number of E- per plants in ranged from above 80 of panicle number accounted for 40.5% of the measured plants (Table 2).

Table 2 The percentage of plants within each category of crown width, plant height and panicle number of E+ and E- plants within three years

Time	Endophyte status	Crown width (cm)					Plant height (cm)					Panicle number				
		8	16	24	32	≥40	0-15	15-30	30-45	45-50	≥ 50	0-20	20-40	40-60	60-80	≥ 80
2015	E+	60.2	23.7	3.1	6.0	7.0	20.7	29.5	32.9	10.8	6.1	48.7	20.9	13.8	6.2	9.4
	E-	40.7	30.6	10.3	16.8	1.6	29.6	36.9	21.3	7.4	4.8	62.2	21.3	8.6	5.4	2.5
2016	E+	33.4	30.8	10.2	9.5	16.1	4.0	20.6	54.1	10.6	10.7	12.0	10.0	23.0	16.8	38.2
	E-	35.3	33.7	10.9	20.7	3.2	15.8	40.9	31.8	7.7	3.8	28.9	23.8	10.9	5.8	30.6
2017	E+	11.3	20.1	18.8	16.9	32.9	3.2	8.7	70.3	12.2	5.6	2.8	6.8	14.2	22.3	53.9
	E-	20.6	26.7	20.6	30.8	1.2	9.8	62.5	20.5	4.9	2.3	16.3	20.2	13.0	10.0	40.5

In 2017, the plant withering stage of E+ plants was 4 and 7 d later than each of the previous two years, respectively, and the green-up stage was 4 and 6 d earlier than the second year and the first year, respectively (Table 3). The plant withering stage in E+ plants was 4d, 5d, and 5d later than in E- plants, and the green-up stage of E+ plants was 2d, 3d, and 6d earlier than E- plants in 2015, 2016, and 2017 (Table 3). The green stage in E+ plants was 233, 238, and 246 days in 2015, 2016, and 2017, respectively, while the green stage in the third year was 8 and 13 days longer than it was in each of the previous two years, respectively, and

was 3d, 4d, and 9 d longer than E- plants (Table 3). The overwintering rates of E+ plants were 78.6%, 80.3%, and 92.2% in 2015, 2016, and 2017, respectively, and were 0.7%, 3.9%, and 10.5% higher than E- plants (Table 3).

Table 3 Overall field performance of E+ and E- perennial ryegrass plants

Year	Withering stage (d/m/y)		Returning green stage (d/m/y)		Green stage (d)		Overwintering rate (%)	
	E+	E-	E+	E-	E+	E-	E+	E-
2014	02/12/2014	29/11/2014	12/04/2015	14/04/2015	233	230	75.6	74.9
2015	06/12/2015	01/12/2015	10/04/2016	13/04/2016	238	234	81.4	77.5
2016	09/12/2016	04/12/2016	06/04/2017	10/04/2017	246	237	92.2	81.7

Growth parameters under different sowing dates

Late sowing had a negative effect on plant growth, as shown by the reduction in the growth parameters recorded for the four varieties under study in the later plantings (Figure 1, Table 5). N plants from the October planting date had longer and thicker roots, and deeper and thicker root necks than F, E and L plants (Figure 1).

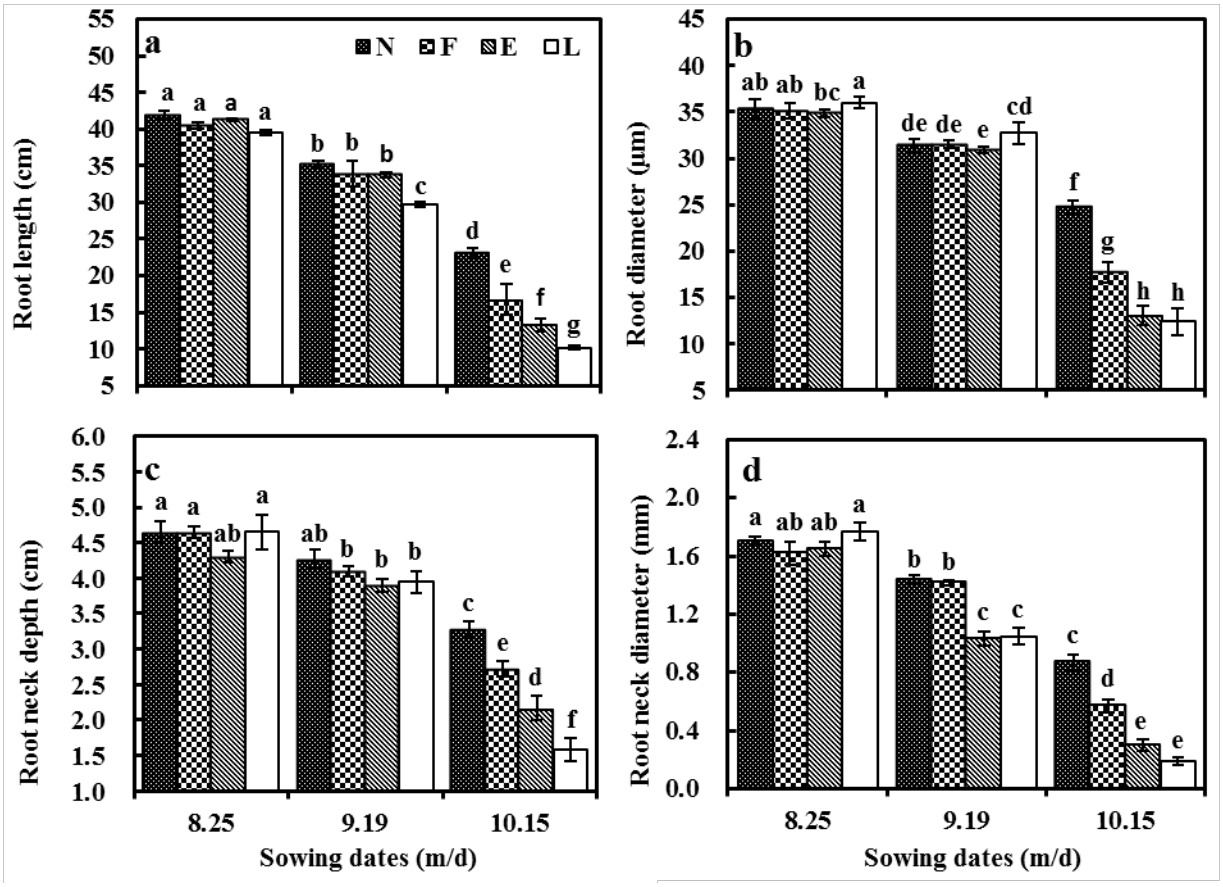

Figure 1. Growth parameters of *Lolium perenne* under different dates of sowing. a: Root length, b: Root diameter, c: Root neck depth, and d: Root neck diameter. N: High endophyte subpopulation, F: The parent population, E: Control subpopulation of endophyte-free, and L: Control variety of local. The values presented are mean ± standard error (SE). Lowercase letters (a, b, c, d, e, f and g) on top of bars indicate significant differences ($P < 0.05$) of N, E, F and L plants under the different date of sowing.

With the delay in the sowing date, the withering stage of N, F, E, and L plants began earlier and there was little difference among the four varieties (Table 4). The green-up stage of the high endophyte subpopulation began significantly earlier among the plants from the October 15th sowing, being 9, 21, and 23 days earlier than among the F, E, and L plants, respectively (Table 4). In addition, the rate of overwintering was significantly higher for the N than for the F, E, and L plants for the August 25 and September 19 sowings (Tables 4 and 5).

Table 4 Plant field performance and overwintering rate of high-endophyte subpopulation (N), the parent population (F), the endophyte-free subpopulation (E), and the local variety (L) of *Lolium perenne* at the withering and regreening stages, planted on three different dates in 2017

Variety	Withering stage (d/m)			Returning green stage (d/m)			Rate of overwintering (%)		
	25/8	19/9	15/10	25/8	19/9	15/10	25/8	19/9	15/10
N	04/12	01/12	25/11	16/4	14/4	28/3	95.7a	92.6b	87.9d
F	05/12	03/12	24/11	17/4	15/4	06/4	93.3b	89.8c	76.2e
E	07/12	02/12	22/11	14/4	17/4	18/4	92.4b	88.6c	69.7f
L	28/11	26/11	21/11	20/4	25/4	20/4	92.6b	87.9c	70.1f

Table 5 Two-way ANOVA for the effects of variety (V) and sowing date (D) on plant height, root length, root neck depth, root neck diameter, and overwintering rate of *L. perenne.*

Source	df	Root length		Root diameter		Root neck depth		Root neck diameter		Over-wintering rate	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
V	3	18.4	<0.001	724.6	<0.001	348.1	<0.001	307.9	<0.001	189.7	<0.001
D	2	958.9	<0.001	26.9	<0.001	14.5	<0.001	17.5	<0.001	50.9	<0.001
V×D	6	24.3	<0.001	7.2	<0.001	14.8	<0.001	7.0	<0.001	98.3	<0.001

F is F-value, statistical value of F-test;

P value is probability greater than the calculated value; significance p ≤ 5%, 1%, and 0.1% levels, respectively

Enzymatic activity with low temperature stress

SOD activity of E+, E- the subpopulations and the parent population

Results showed that SOD activity increased with decreasing temperature (Table 6, Figure 2). Further, SOD activity was significantly higher in E+ plants of the subpopulations than in the mixed E+ and E- plants of the parent population in the 0, 5, and 10 °C, treatment groups; however, the SOD activity of E- plants of the subpopulations and of the parent did not differ from one another; lastly, SOD activity was higher in E+ than in E- plants of the subpopulations at 0, 5, and 10 °C; however, there was no difference between E+ and E- plants of the parent population under cold stress (Figure 2).

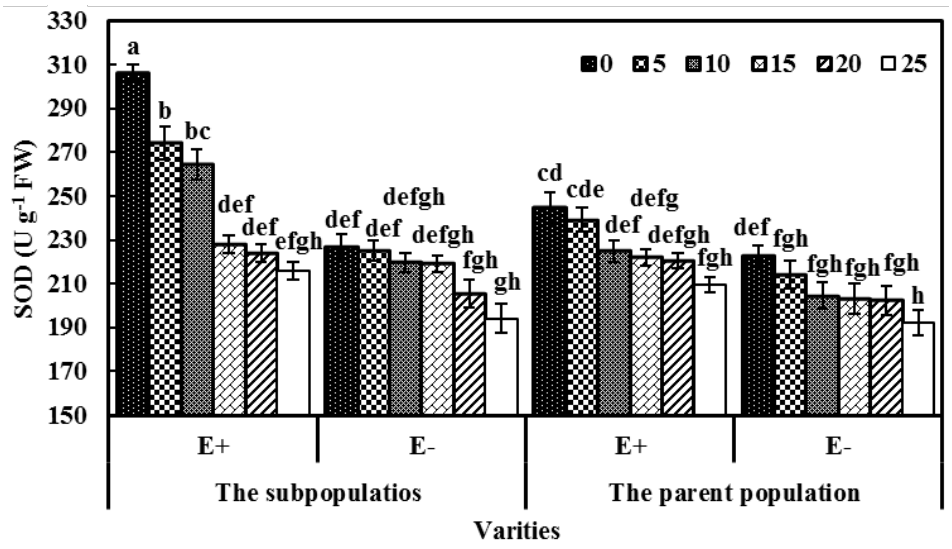

Figure 2. SOD activity in endophyte-infected (E+) and -free (E-) *Lolium perenne* plants of the subpopulations and the parent population with low temperature stress. The values presented are mean ± standard error (SE). Lowercase letters (a, b, c, d, e, f, g, and h) on top of bars indicate significant differences ($P < 0.05$) of the subpopulations (E+ and E-) and the parent population (E+ and E-) under the different temperatures.

SOD activity of E+ and E− plants in stems, roots, and root hairs

The presence of endophytic fungi resulted in higher activity of SOD in stems, roots, and root hairs than in those plants without these fungi (Table 6, Figure 3), being 8.6%, 9.2%, and 14.4% higher than in stems, roots, and root hairs of E- plants, respectively (Figure 3); SOD activity of endophyte-free plants was significantly different among stems, roots, and root hairs, but E+ plants did not show significant difference in SOD activity between the stem and root ($P = 0.604$) (Figure 3). In addition, SOD activity of endophyte-infected plants was higher in stems and roots than in root hairs (Figure 3).

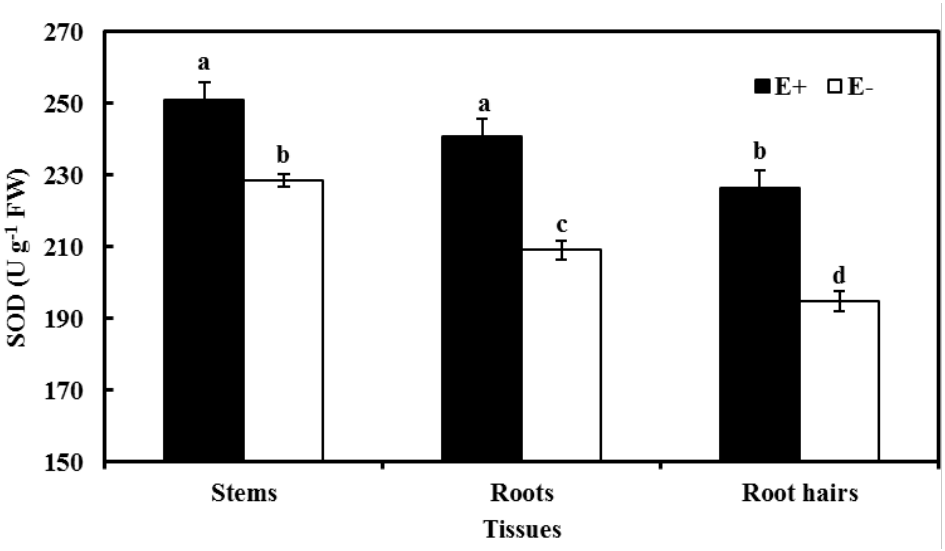

Figure 3. SOD activity in stems, roots, and root hairs of endophyte-infected (E+) and -free (E-) *Lolium perenne* plants. The values presented are mean ± standard error (SE). Lowercase letters (a, b, c, and d) on top of bars indicate significant differences ($P < 0.05$) of between E+ and E- plants.

E+ and E- POD activity of the subpopulations and the parent population

Comparisons of the subpopulations and the parent population under low temperature stress showed that the source of different seeds significantly influenced plant POD activity (Table 6), as E+ plants of the subpopulation showed higher POD activity than the E+ plants of the parent population at 0, 5, and 10 °C (Figure 4). Within the range of temperatures tested, POD activity in the E+ plants of the subpopulation was consistently higher than that in E- plants of the parent population (Figure 4). Further, our results showed that *Lolium perenne* plants of the subpopulations showed significant differences in POD activity at 0, 5, and 10 °C, while E+ and E- plants of the parent population were significantly different in POD activity only at 0 °C (Table 6, Figure 4).

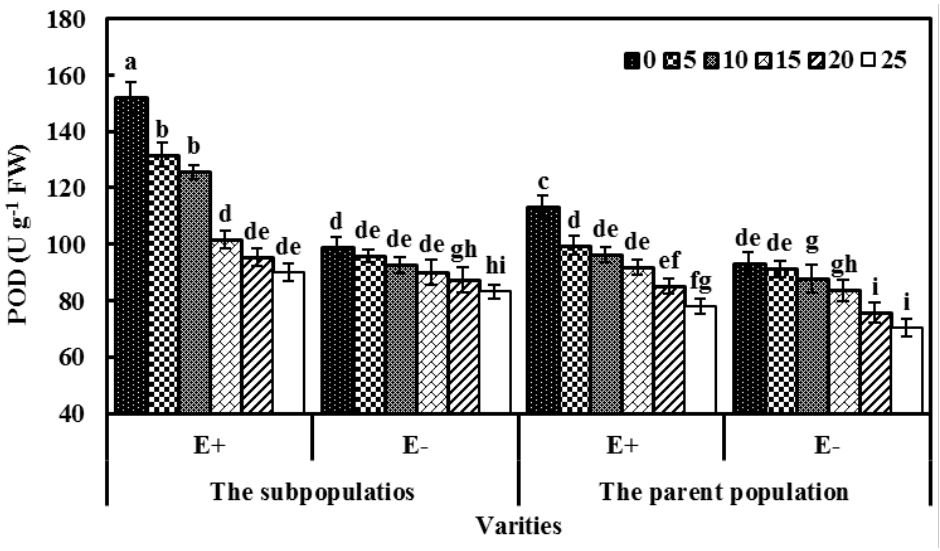

Figure 4. POD activity in endophyte-infected (E+) and -free (E-) *Lolium perenne* plants of the subpopulations and the parent population with low temperature stress. The values presented are mean ± standard error (SE). Lowercase letters (a, b, c, d, e, f, g, h, and i) on top of bars indicate significant differences ($P < 0.05$) of the subpopulations (E+ and E-) and the parent population (E+ and E-) under the different temperatures.

E+ and E- CAT activity of the subpopulations and the parent population

As observed for SOD activity, CAT activity increased as temperature decreased (Table 6, Figure 5). There were significant differences in temperature effects on the CAT activity between the subpopulations and the samples from the parent population: the CAT activity in the E+ of the subpopulation was generally higher than that in the E+ of the parent population under mild cold stress conditions (0, 5, and 10 °C) (Table 6, Figure 5). CAT activity was higher in E+ than E− plants of the subpopulations under low-temperature stresses (0, 5, and 10 °C), but not in the parent population (Figure 5).

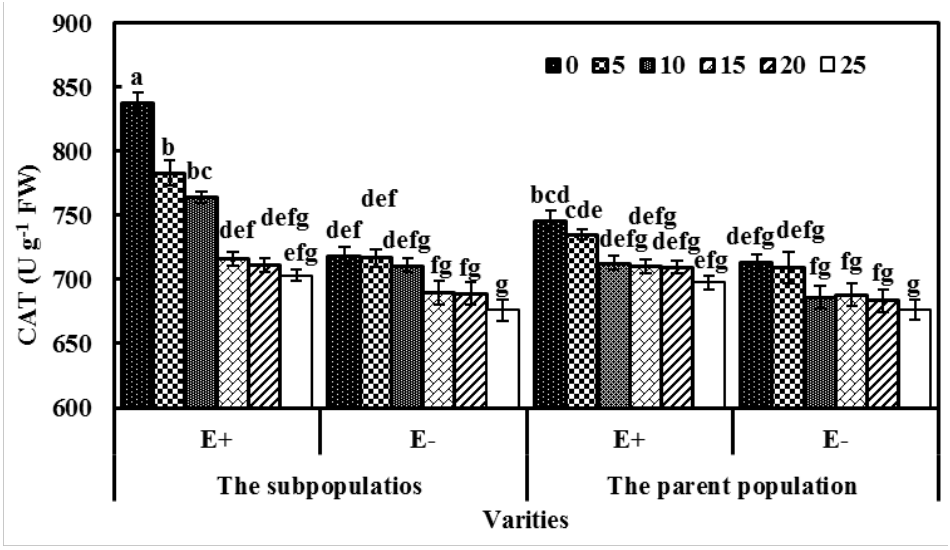

Figure 5. CAT activity in endophyte-infected (E+) and -free (E-) *Lolium perenne* plants of the subpopulations and the parent population with low temperature stress. The values presented are mean ± standard error (SE). Lowercase letters (a, b, c, d, e, f, and g) on top of bars indicate significant differences ($P < 0.05$) of the subpopulations (E+ and E-) and the parent population (E+ and E-) under the different temperatures.

CAT activity in stems, roots, and root hairs of E+ and E− plants

The presence of the endophyte positively related with CAT activity in stems, roots, and root hairs, compared to endophyte-free plants (Table 6, Figure 6). Additionally, CAT activity in E+ plants increased by 6.5%, 5.5%, and 7.4% in the stems, roots and root hairs, compared with that in E− plants, respectively (Table 6, Figure 6). Further, there were significant differences in CAT activity among stems, roots, and root hairs of endophyte-free plants (Figure 6), but CAT activity was not significantly higher in roots or root hairs ($P = 0.714$), while SOD activity in roots and root hairs of E+ plants was lower than in stems (Table 6, Figure 6).

Figure 6. CAT activity in stems, roots, and root hairs of endophyte-infected (E+) and -free (E-) *Lolium perenne* plants. The values presented are mean ± standard error (SE). Lowercase letters (a, b, c, and d) on top of bars indicate significant differences ($P < 0.05$) of between E+ and E- plant.

APX activity in E+ and E- plants of the subpopulations and in the parent population

The APX activity increased as the temperature declined in both the subpopulations and the parent population of *Lolium perenne* (Table 6, Figure 7). Under lower temperature stress, a higher APX activity in the E+ plants of the subpopulation was

found when compared to E+ or E- plants of the parent population, and significant main effects were present at 0, 5, and 10 °C (Table 6, Figure 7). Further, the presence of the endophyte increased APX activity, especially under low temperature stress (Figure 7). At 0, 5, and 10 °C, significantly higher APX activity was recorded in E+ plants of the subpopulation than in E- plants (Figure 7). For the parent plants, the APX activity of E+ plants was significantly higher than that of E- plants at 0 °C (Figure 7).

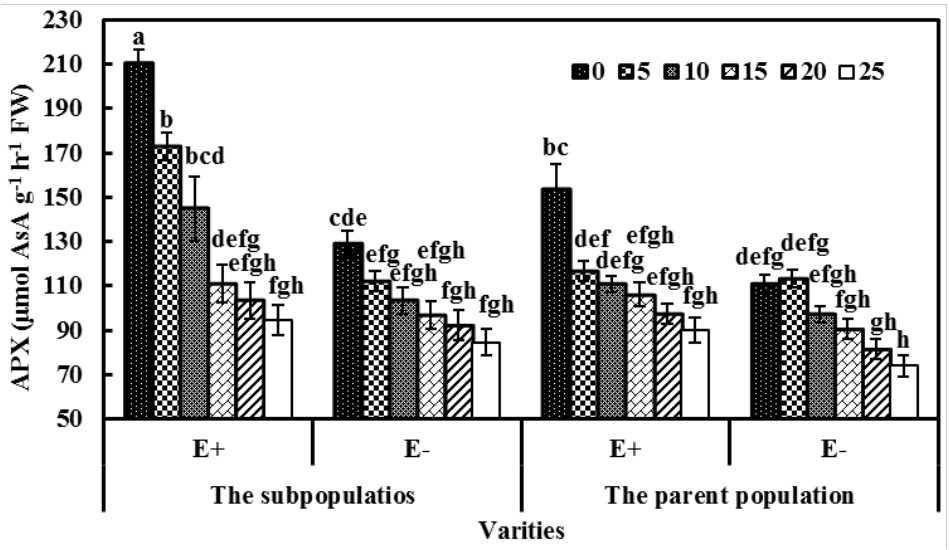

Figure 7. APX activity in endophyte-infected (E+) and -free (E-) *Lolium perenne* plants of the subpopulations and the parent population with low temperature stress. The values presented are mean ± standard error (SE). Lowercase letters (a, b, c, d, e, f, and g) on top of bars indicate significant differences ($P < 0.05$) of the subpopulations (E+ and E-) and the parent population (E+ and E-) under the different temperatures.

APX activity in stems, roots, and root hairs of the subpopulations and of the parent population

The APX activity was not significantly different between the stems and roots of the subpopulations and the parent, but the root hairs had higher the APX activity in the subpopulations than in the parent (Table 6, Figure. 8).

Figure 8. APX activity in stems, roots, and root hairs of *Lolium perenne* plants of the subpopulations and the parent population. The values presented are mean ± standard error (SE). Lowercase letters (a, b, and c) on top of bars indicate significant differences ($P < 0.05$) of stems, roots and root hairs of the subpopulations and the parent population. The values presented are mean ± standard error (SE).

Table 6 Four-way ANOVA for the effects of variety (V), endophyte status (E_1), stress level (S) and tissue (T) on SOD, POD, CAT, and APX activities in plants of *Lolium perenne*

Source	df	SOD		POD		CAT		APX	
		F	*P*	F	*P*	F	*P*	F	*P*
V	1	102.1	<0.001	177.6	<0.001	89.6	<0.001	72.6	<0.001
E_1	1	305.0	<0.001	432.4	<0.001	333.9	<0.001	171.5	<0.001
S	5	76.9	<0.001	118.8	<0.001	95.6	<0.001	92.9	<0.001
T	2	109.6	<0.001	66.8	<0.001	123.5	<0.001	62.8	<0.001
V×E	1	23.1	<0.001	14.2	<0.001	35.1	<0.001	19.5	<0.001
V×S	5	9.0	<0.001	3.3	0.008	13.6	<0.001	6.0	<0.001
V×T	2	0.7	<0.001	0.5	0.583	0.1	0.922	3.6	0.030
E_1×S	5	12.0	<0.001	3.9	0.003	15.8	<0.001	13.8	<0.001
E_1×T	2	3.6	0.028	1.3	0.290	6.9	0.001	1.1	0.347
S×T	10	0.3	0.951	0.3	0.978	0.4	0.958	3.8	0.452
V×E_1×S	5	8.7	<0.001	7.7	<0.001	10.8	<0.001	6.4	<0.001
V×E_1×T	2	0.2	0.824	0.6	0.556	0.3	0.767	0.8	0.456
V×S×T	10	0.2	0.995	0.3	0.969	0.5	0.911	0.9	0.579
E_1×S×T	10	0.6	0.845	0.6	0.810	0.7	0.755	1.0	0.487
S×E_1×S×T	10	0.3	0.970	0.2	0.998	0.2	0.993	0.4	0.938

Discussion

This study is one of the few studies on cold tolerance in the perennial ryegrass-*E. festucae* var. *lolii* symbiosis in recent years. Our systematic approach included the selection of a high-endophyte subpopulation, field evaluation, and the study of the possible mechanisms underlying the cold tolerance of the high endophyte subpopulation. The high endophyte subpopulation was segregated from the parent ryegrass cultivar via plant selection over 3 years. The high-endophyte subpopulation showed improved agronomic traits and showed high winter hardiness in the field; this may be attributed to its improved root system and over-wintering ability, as well as to the enhanced levels of ROS-scavenging enzyme activities in both the above and belowground plant organs.

The high-endophyte subpopulation had high infection rates and improved agronomic traits

The presence of endophyte-produced alkaloids improves host resistance to insects (Hennessy et al., 2016), rodents (Conover, 2003), birds (Pennell et al., 2010), and diseases (Kelemu et al., 2004) and are therefore useful in turfgrass cultivars. Some studies have shown that turfgrass cultivars with high endophyte infection rates had higher alkaloid contents (ergovaline and lolitrem B)

than cultivars with low infection rates by endophytes (Maejima et al., 2000). High endophyte infection rates can increase competitive abilities of host plants in natural plant communities (Brem and Leuchtmann, 2010). Żurek et al. (2012) showed that endophyte infection rates in seeds increased with the postponement of the sowing year, which is consistent with our observations of endophyte infection rates above 95% in tillers and seeds after three years of planting and screening.

Previous studies indicated that infection with endophytes could impart an agronomic advantage to host plants (Hume et al., 2009; Hume et al., 2015). For example, infected plants show increased tiller number (Murphy et al., 2015), survival (Wheatley et al., 2007), biomass (Richmond et al., 2006) and productivity (Young et al., 2013) over time, compared with endophyte-free plants. The results of the present study support these findings. High endophyte infection rate improved agronomic traits (crown width, plant height, ear number, withering stage, returning green stage, green stage, and overwintering rates). Panicle number was the main driver of the increase in biomass and seed yield (Murphy et al., 2015). Crown width, green stage and withering stage are important evaluation indices of turfgrass quality, and the level of these indicators directly determines the utilization value of turfgrass varieties (Narra, 2007). Winter survival rates can serve as an effective measure to evaluate the low temperature injury and adaptability of plants (Wang et al., 2012). The combination of improved agronomic traits in one single plant is considered the determinant of parental variety quality for plant breeding purposes (Barakat et al., 2013).

The high-endophyte subpopulation contained plants with high cold tolerance, well-developed root systems, and high winter survival rates

Low temperature stress not only leads to a decrease in the distribution and survival of the plant but also causes plant death when severe freezing occurs (Clarke and Siddique., 2004; Mantri et al., 2010; Xin and Browse, 2010); especially at the seedling and flowering stages, and is the main reason for slow establishment rates, low seed set, and poor crop yield (Sanghera et al., 2011). In cold-tolerant varieties, the key factors that determine the level of cold tolerance in alfalfa include root characteristics (Svačina et al., 2014). Similarly, a previous study showed significant differences in the number of lateral roots in alfalfa varieties and strains between those with high and low cold tolerance (Smith, 1951). Root necks and roots were thicker in the cold tolerant alfalfa varieties, and alfalfa plants with thicker root systems (root diameter > 10 mm) suffered less damage than alfalfa plants with smaller root diameters (root diameter < 1-5 mm) (Yves et al., 2009). The findings of the present study showed that the high

infection of plants resulted in significantly thicker diameters in their root necks than plants in the populations with lower infection levels (F, E, and L) at the October 15 planting, while root length and root neck depths were significantly higher in the high-endophyte subpopulation than in F, E, and L. Thus, the high endophyte infection rate was consistently associated with improved cold resistance of the host plants.

Plant regreening stage is the key period for development of new tillers and root formation in the spring (Kong et al., 2012). Our results showed that the presence of *E. festucae* var. *lolii* in the *L. perenne* subpopulation shortened the time before the regreening of plants. The stage from the beginning of plant greening-up to the jointing stage is an important stage for vegetative growth and is also the basis for the reproductive growth of panicle differentiation, and a short period prior to the green-up stage was beneficial for the absorption of sufficient water and nutrients to nourish active vegetative growth (Shao et al., 2013). Plant cold tolerance varies significantly between different ecotypes or landraces, and the winter survival rate is the most commonly employed measure for estimating plant cold hardiness (Brulebabel and Fowler., 1989). A similar result was found in the present study, where the high-endophyte subpopulation had a higher rate of successful overwintering than the parent, the endophyte–free subpopulation, and the local variety. This finding suggested a positive effect of the high infection rate of endophyte on the cold tolerance of the host plants, consistently with results reported by Chen et al. (2008) for *Achnatherum inebrians*.

The high-endophyte subpopulation showed higher SOD, POD, CAT, and APX activities

Under low temperature stress, the first mechanisms to consider in relation to tolerance are the enzyme activities of the plants. The antioxidant system in plants is crucial for the removal of excess ROS induced by the stress (Lee and Lee., 2000). A previous study showed that one of the most determinant cold-tolerance traits is the capacity of SOD, POD, CAT, and APX enzymes to maintain their activities during low temperature in cold-resistant cultivars (Lukatkin., 2002; Hashempour et al., 2014). The findings of the present study showed that E+ plants of the high-endophyte subpopulation in fact did show higher activity of SOD, POD, and CAT, than the E+ plants of the parent at 0, 5, and 10 °C, while APX activity was higher at 0 and at 5 °C, which was consistent with the findings of Hill et al. (1990) and Zhang et al. (2013). Some studies on tall fescue, fine fescue, drunken horse grass, and perennial ryegrass have shown that endophyte infection can promote antioxidant enzyme activity and nonenzymatic antioxidants content under stress (Chen et al., 2008; Hamilton et al., 2012; Kari et al., 2013; Ma et al., 2015; Zhang et al., 2010).

Similarly, the results of the present study showed that *Epichloë* infected plants had increased POD and APX activity in the parent plants, and increased SOD, POD, CAT, and APX activity in plants of the high-endophyte subpopulation under LTS. In winter, the aboveground plant parts withered and yellowed, while the vigor of stems and roots reflects cold hardiness (Liu and Yu., 2009). Endophytes show a gradient distribution trend for concentration from high to low and from bottom to top, so that the lowest concentration of *Epichloë* was found in leaves of the grass, while the highest concentration was detected in stem pulp (Herd et al., 1997; Siegel et al., 1990). Consistently, SOD and CAT activities in *Epichloë*-infected *L. perenne* plants ranked in the following decreasing order: stems, roots, and root hairs, which was similar to the findings of Chen et al. (2008).

Stems, roots, and root hairs of the high-endophyte subpopulation show higher APX activity

Higher APX activity enhanced salt tolerance in onion plants (Orabi et al., 2010) and in sugar beet (Dadkhah., 2008). The activities of antioxidant enzymes (SOD, POD, CAT, and APX) remarkably increased to eliminate excess ROS accumulation induced by LTS (Kang et al., 2003) and maintained the ascorbate pool, which indirectly enhanced plant tolerance under LTS. Previous studies have shown that the same endophyte-infected host plants under the same environmental conditions, will vary in response not only to host species and variety, but also to different genotypes of the same variety (Kover et al., 1997). Numerous studies have shown that *Epichloë* infection enhances host tolerance to abiotic and biotic stress conditions (Barnawal et al., 2016; Chadha et al., 2015; Rho et al., 2017). Hill et al. (1990) reported that the presence of one kind of endophyte may increase the tolerance of a host species to some extent, but may have no effect on other hosts of the same species under the same conditions. The findings of the present study showed that APX activity in the stems, roots, and root hairs of the plants of the high-wndophyte subpopulation was higher than that in the same organs of the parent plants. It has also been shown that infection of different strains of *Epichloë* significantly influenced phenolic content and antioxidant activity of perennial ryegrass, having no effect, positive effects, or negative effects on phenol content, depending on the endophyte strain (Qawasmeh et al., 2012).

Conclusions

In this study, we obtained a high-endophyte subpopulation with high endophyte infection rates in tillers and seeds (≥95%) and improved agronomic traits. Further, we found that the high-endophyte endophyte subpopulation showed good cold tolerance with well-developed roots and root necks, high winter survival rate, with up-regulated SOD, POD, CAT, and APX activities. Collectively, our findings provide a theoretical basis for the cultivation of high endophyte cultivars of perennial ryegrass to improve grass performance in northern China, especially in the Qinghai-Tibet Plateau.

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Conflict of interest

The authors declare that they have no competing interests.

References

Barakat MN, Aldoss AA, Elshafei AA, Ghazy AI, Moustafa KA (2013) Assessment of genetic diversity among wheat doubled haploid plants using TRAP markers and morpho-agronomic traits. Aust J Crop Sci 7:104-111. https://doi.org/10.1007/BF00046401

Barnawal D, Bharti N, Tripathi A, Pandey SS, Chanotiya CS, Kalra A, (2016) ACC-deaminase-producing endophyte brachybacterium paraconglomeratum strain SMR20 ameliorates chlorophytum salinity stress via altering phytohormone generation. J Plant Growth Reg 35:1-12. https://doi.org/10.1007/s00344-015-9560-3

Barrero-Gil J, Huertas R, Rambla JL, Granell A, Salinas J (2016) Tomato plants increase their tolerance to low temperature in a chilling acclimation process entailing comprehensive transcriptional and metabolic adjustments. Plant Cell Environ 39:

2303-2318. https://doi.org/10.1111pce.12799

Bilska A, Sowiński P (2010) Closure of plasmodesmata in maize (*Zea mays*) at low temperature: a new mechanism for inhibition of photosynthesis. Ann Bot-london 106:675-686. https://doi.org/10. 1093/aob/mcq169

Bhowmik PK, Tamura K, Sanada Y, Yamada T (2006) Sucrose metabolism of perennial ryegrass in relation to cold acclimation. Z Naturforsch C 61:99-104. https://doi.org/10.1515/znc-2006-1-218

Brem D, Leuchtmann A (2010) Intraspecific competition of endophyte infected vs uninfected plants of two woodland grass species. Oikos 96:281-290. https://doi.org/10.1034/j.1600- 0706.2002.960210.x

Brulebabel AL, Fowler DB (1989) Use of controlled environments for winter cereal cold hardiness evslua. Can J Plant Sci 69: 355-366. https://doi.org/10.4141 /cjps89-047

Casler MD, Santen EV (2008) Fungal endophyte removal does not reduce cold tolerance of tall fescue. Crop Sci 48:2033-2039. http://dx.doi.org/10.2135/cropsci2007.11.0615

Chadha N, Mishra M, Rajpal K, Bajaj R, Choudhary DK, Varma A (2015) An ecological role of fungal endophytes to ameliorate plants under biotic stress. Arch Microbiol 197:869-881. https:// doi.org/10.1007/s00203-015-1130-3

Chen N (2008) Genetic diversity of drunken horse grass (*Achnatherum inebrians*) and effects of its endophyte infection on cold tolerance. Thesis (Ph.D.): LanZhou University. (In Chinese, with English abstract)

Chen N, He RL, Chai Q, Li CJ, Nan ZB (2016) Transcriptomic analyses giving insights into molecular regulation mechanisms involved in cold tolerance by *Epichloë* endophyte in seed germination of *Achnatherum inebrians*. Plant Growth Regu 80: 367-375. https://doi.org/10.1007/ s10725-016-0177-8

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 428:1-18.https://doi.org/10.1007/ s11104-018-3643-4

Chen Y, Jiang JF, Chang QC, Gu CS, Song AP, Chen SM, Dong B, Chen FD (2014) Cold acclimation induces freezing tolerance via antioxidative enzymes, proline metabolism and gene expression changes in two chrysanthemum species. Mol Biol Rep 41:815-822. https://doi.org/10.1007/s11033-013-2921-8

Chen ZJ, Chen H, Wei XK, Tian P, Li CJ, Nan ZB (2018b) Screening of individual plants of *Lolium perenne* with high endophyte infection rate.10th International Symposium on Fungal Endophytes of Grasses:Book of abstracts 77. http://isfeg2018. fundacionusal.es/

Chen ZJ, Wei XK, Ying C, Tian P, Zhao XJ, Li CJ (2017) Research progress of methods on grass fungal endophyte detection. Pratacultural Science 11:1419-1433. (In Chinese, with English abstract)

Christensen MJ, Bennett RJ, Ansari HA, Koga H, Johnson RD, Bryan GT, Simpson WR, Koolaard JP, Nickless EM, Voisey CR (2008) *Epichloë* endophytes grow by intercalary hyphal extension in elongating grass leaves. Fungal Genetics Biology Fg

B 45:84-93. https://doi.org/10.1016/j.fgb.2007.07.013

Clarke HJ, Siddique K (2004) Response of chickpea genotypes to low temperature stress during reproductive development. Field Crops Res 90:323-334. https://doi.org/10.1016/j.fcr.2004.04. 001

Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. American Naturalist 160:S99-S127. https://doi.org/10.1086/342161

Conover MR (2003) Impact of the consumption of endophyte-infected perennial ryegrass by meadow voles. Agr Ecosyst Enviro 97:199-203. https://doi.org/10.1016/S0167-8809(03)00037-9

Dadkhah A (2008) Response of root yield and quality of sugar beet (*Beta vulgaris*) to salt stress. Comp Biochem Phys 150:S196-S203. https://doi.org/10.1016/j.cbpa.2008.04.541

Dalmannsdottir S, Jørgensen M, Rapacz M, Østrem L, Larsen A, Rødven R, Rognli OA (2017) Cold acclimation in warmer extended autumns impairs freezing tolerance of perennial ryegrass (*Lolium perenne*) and timothy (*Phleum pratense*). Physiol Plant 160:266-281. https://doi.org/10.1111/ppl.12548

Faltusová-Kadlecová Z, Faltus M, Prášil I (2002) Comparison of barley response to short-Term cold or dehydration. Biol Plantarum (Prague) 45:637-639. https://doi.org/10.1023/A:1022353316419

Florea S, Schardl CL, Hollin W (2015) Detection and isolation of *Epichloë* species, fungal endophytes of grasses.Curr Protoc Microbiol 38:19A.1-24. https://doi.org/10.1002/9780471729259.mc19a01s38

Garcíalara S, Arnason JT, Díazpontones D, Gonzalez E, Bergvinson DJ (2007) Soluble peroxidase activity in maize endosperm associated with maize weevil resistance. Crop Sci 47:1125-1130. http://dx.doi.org/10.2135/cropsci2006.10.0687

Gómez LD, Vanacker H, Buchner P, Noctor G, Foyer CH (2004) Intercellular distribution of glutathione synthesis in maize leaves and its response to short-term chilling. Plant Physiol 134:1662-1671. https://doi.org/10.1104/pp.103.033027

Gundel PE, Rudgers JA, Whitney KD (2017) Vertically transmitted symbionts as mechanisms of transgenerational effects. Am J Bot 104:787-792. https://doi.org/10.3732/ajb.1700036

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

Hashempour A, Ghasemnezhad M, Ghazvini RF, Sohani M (2014) Olive (*Olea europaea* L.) freezing tolerance related to antioxidant enzymes activity during cold acclimation and non acclimation. Acta Physiol Plant 36:3231-3241. https://doi.org/10.1007/s1173-014-1689-3

Han H, Han B (2015) Evaluation of cold resistance and selection of chill-proof measure of three *Phlox* cultivars. Journal of Anim Plant Sci 25:208-212.

Hennessy LM, Popay AJ, Finch SC, Clearwater MJ, Cave VM (2016) Temperature and plant genotype alter alkaloid concentrations in ryegrass infected with an *Epichloë* endophyte and this affects an insect herbivore. Front Plant Sci 7:1907-1917. https://doi.org/10.3389/fpls.2016.01097

Herd S, Christensen, MJ, Saunders K, Scott DB, Schmid J (1997) Quantitative assessment of in planta distribution of metabolic activity and gene expression of an endophytic fungus. Microbiology 143: 267-275. https://doi.org/10.1007/978--4899-0271-9_8

Hill NS, Stringer WC, Rottinghaus GE, Belesky DP, Parrott WA, Pope DD (1990) Growth, morphological and chemical component responses of tall fescue to *Acremonium coenophialum*. Crop Sci 30:239-55. https://www. crops.org/publications cs/abstracts/30/1/CS0300010156

Huang CP, Qin NN, Sun L, Yu MY, Hu WZ, Qi ZY (2018) Selenium improves physiological parameters and alleviates oxidative stress in strawberry seedlings under low-temperature stress. Int J Mol Sci 19:1913-1926. https://doi.org/10.3390/ ijms19071913

Hume DE, Cooper BM, Panckhurst KA (2009) The role of endophyte in determining the persistence and productivity of ryegrass, tall fescue and meadow fescue in Northland. Proceedings of the New Zealand Grassland Association 71:145-150.

Hume DE, Roodi D, Mcgill CR, Millner JP, Johnson RD (2015) Beneficial endophytic microorganisms of *Brassica* – A review. Biol Control 90:102-112. https://doi.org/10.1016/j.biocontrol.2015.06.001

Hund A (2010) Genetic variation in the gravitropic response of maize roots to low temperatures. Plant Root 4:22-30.https://doi. org/10.3117/plantroot.4.22

Hund A, Fracheboud Y, Soldati A, Stamp, P (2008) Cold tolerance of maize seedlings as determined by root morphology and photosynthetic traits. Eur J Agron 28:178-185. https://doi.org/10.1016/j.eja.2007.07.003

İşeri ÖD, Körpe DA, Sahin FI, Haberal M (2013) Hydrogen peroxide pretreatment of roots enhanced oxidative stress response of tomato under cold stress. Acta Physiol Plantarum 35:1905-1913. https://doi.org/10.1007/s11738-013-1228-7

Jha UC, Bohra A, Jha R (2017) Breeding approaches and genomics technologies to increase crop yield under low-temperature stress. Plant Cell Rep 36:1-35. https://doi.org/10.1007/s00299-016-2073-0

Kang GZ, Wang CH, Sun GC, Wang ZX (2003) Salicylic acid changes activities of H2O2-metabolizing enzymes and increases the chilling tolerance of banana seedlings. Environ Exp Bot 50:9-15. https://doi.org/10.1016/S0098-8472(02)00109-0

Kari S, Gundel PE, Marjo H (2013) Chemical ecology mediated by fungal endophytes in grasses. J Chem Ecol 39:962-968. https://doi.org/10.1007/s10886-013-0310-3

Kelemu S, Cardona C, Segura G (2004) Antimicrobial and insecticidal protein isolated from seeds of Clitoria ternatea, a tropical forage legume. Plant Physiol Bioch 42:867-873. https://doi.org/10.1016/j.plaphy.2004.10.013

Kong LY, Yan H, Bao YS, Chen HL (2012) Remote sensor monitoring method for winter wheat growth based on key development periods. Chinese Journal of Agrometeorology 33:424-430. (In Chinese, with English abstract)

Kover PX, Dolan TE, Clay K (1997) Potential versus actual contribution of vertical transmission to pathogen fitness. P Royl Soc B-Biol Sci 264:903-909. https://doi.org/10.1098/rspb.1997.0125

Kovi MR, Fjellheim S, Sandve SR, Larsen A, Rudi H, Asp T, Kent MP, Rognli OA (2015) Population structure, genetic variation,

and linkage disequilibrium in perennial ryegrass populations divergently selected for freezing tolerance. Front Plant Sci 6: 929-942. https://doi.org/10.3389/fpls.2015.00929

Lee BH, Lee HJ, Xiong LM, Zhu JK (2002) A mitochondrial complex I defect impairs cold-regulated nuclear gene expression. Plant Cell 14:1235-1251. https://doi.org/10.1105/tpc.010433

Lee DH, Lee CB (2000) Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. Plant Sci 59:75-85. https://doi.org/10.1016/S0168-9452(00)00326-5

Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim JH (2003). Screening of medicinal plant extracts for antioxidant activity. Life Sci, 73:167-179. https://doi.org10.1016/S0024-3205(03)00259-5

Leuchtmann A, Bacon CW, Schardl CL, White JF, Tadych M (2014) Nomenclatural realignment of *Neotyphodium* species with genus *Epicholë*. Mycologia 106:202-215. https://doi.org/10.3852/13-251

Li BQ, Zhang CF, Cao BH, Qin GZ, Wang WH, Tian, SP (2012) Brassinolide enhances cold stress tolerance of fruit by regulating plasma membrane proteins and lipids. Amino Acids 43:2469- 2480. https://doi.org/10.1007/s00726-012-1327-6

Liu BY, Lei CY, Shu T, Zhang YS, Jin JH, Li S, Liu WQ (2015) Effects of low-temperature stress on secondary metabolism in mosses exposed to simulated N deposition. Transactions of the Botanical Society of Edinburgh: 8:415-426. https://doi.org/10.1080/17550874.2015.1010187

Liu CH, Yu D (2009) The bud and root sprouting capacity of *Alternanthera philoxeroides* after over-wintering on sediments of a drained canal. Hydrobiologia 623:251-256. https://doi.org/10.1007/s10750-008-9693-5

Lukatkin A S (2002) Contribution of oxidative stress to the development of cold-induced damage to leaves of chilling-sensitive plants: 2. The Activity of Antioxidant Enzymes during Plant Chilling. Russ J Plant Physhl+ 49:782-788. https://doi.org/10.1023/A:1020965629243

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. https://doi.org/10.1007/s10658-014-0563-x

Ma Q, Yue LJ, Zhang JL, Wu GQ, Bao AK, Wang SM (2012) Sodium chloride improves photosynthesis and water status in the succulent xerophyte *Zygophyllum xanthoxylum*. Tree Physiol 32:4–13. https://doi.org/10.1093/treephys/tpr098

Maejima A, Saiga S, Inoue T, Tsuiki M (2000) Endophyte infection rate and alkaloid concentrations in seeds of commercial cultivars of perennial ryegrass. Japanese Journal of Grassland Science 46:52-57.

Mantri NL, Ford R, Coram TE, Pang ECK (2010) Evidence of unique and shared responses to major biotic and abiotic stresses in chickpea. Environ Exp Bot 69:286-292. https://doi.org/10.1016/j.envexpbot.2010.05.003

Malinowski DP, Belesky DP (2010) Ecological importance of *Neotyphodium spp*. grass endophytes in agroecosystems. Grassland Science 52:1-14. https://doi.org/10.1111/j.1744-697X.2006.00041.x

Megha S, Basu U, Kav NNV (2017) Regulation of low temperature stress in plants by microRNAs. Plant, Cell and Environment, 41, 1–15. https://doi.org/10.1111/pce.12956

Mohammadian, MA, Largani ZK, Sajedi RH (2012) Quantitative and qualitative comparison of antioxidant activity in the flavedo tissue of three cultivars of citrus fruit under cold stress. Aust J Crop Sci 6:402-406.

Molinier J, Ries G, Zipfel C, Hohn B (2006) Transgeneration memory of stress in plants. Nature 442:1046-1049. https://doi.org/10.1038/nature05022

Munshaw GC, Ervin EH, Beasley JS, Shang C, Zhang X, Parrish DJ (2010) Effects of late-season ethephon applications on cold tolerance parameters of four bermudagrass cultivars. Crop Sci 50:1022-1029. http://dx.doi.org/10.2135/cropsci2008.09.0565

Murphy B, Martin-Nieto L, Doohan F, Hodkinson T (2015) Fungal endophytes enhance agronomically important traits in severely drought-stressed barley. J Agron Rop Sci 201:419-427. https://doi.org/10.1111/jac.12139

Narra S (2007) Evaluation of sensing and machine vision techniques in stress detection and quality evaluation of turfgrass species. Thesis (Ph.D.): University of Illinois at Urbana-Champaign. http://hdl.handle.net/2142/83121

Orabi SA, Salman SR, Shalaby MAF (2010) Increasing resistance to oxidative damage in cucumber (*Cucumis sativus* L.) plants by exogenous application of salicylic acid and paclobutrazol. World Journal of Agricultural Sciences 6:252-259.

Patchett B, Gooneratne R, Fletcher L, Chapman B (2011) Seasonal changes in leaf and stem loline alkaloids in meadow fescue. Crop Pasture Sci 62:261-267. https://doi.org/10.1071/cp10266

Pennell C, Rolston MP, Bonth AD, Simpson WR, Hume DE (2010) Development of a bird-deterrent fungal endophyte in turf tall fescue. New Zeal J Agr Res 53:145-150. https://doi.org/10.1080/00288231003777681

Qawasmeh A, Obied HK, Raman A, Wheatley W (2012) Influence of fungal endophyte infection on phenolic content and antioxidant activity in grasses: Interaction between *Lolium perenne* and different srains of *Neotyphodium lolii*. J Agr and Food Chem 60:3381-3388. http://dx.doi.org/10.1021%2Fjf204105k

Rasmussen S, Parsons AJ, Newman JA (2009) Metabolomics analysis of the *Lolium perenne–Neotyphodium lolii* symbiosis: more than just alkaloids? Phytochem Rev 8:535-550. https://doi .org/10.1007/s11101-009-9136-6

Redman RS, Yong OK, Woodward CJDA, 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: e14823-e14833. https://doi. org /10.1371/journal.pone.0014823

Rho H, Hsieh M, Kandel SL, Cantillo J, Doty SL, Kim SH (2017) Do endophytes promote growth of host plants under stress? A meta-analysis on plant stress mitigation by endophytes. Microb Ecol 75:1-12. https://doi.org/10.1007/s00248-017-1054-3

Richmond DS, Cardina J, Grewal PS (2006) Influence of grass species and endophyte infection on weed populations during establishment of low-maintenance lawns. Agr Ecosyst Environ 115:27-33. https://doi.org/10.1016/j.agee.2005.12.005

Rodriguez R, Redman R (2007) More than 400 million years of evolution and some plants still can't make it on their own: plant

stress tolerance via fungal symbiosis. J Expl Bot 146:1109-1114. https://doi.org/10.1016/j.cbpa.2007.01.484

Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: A continuum of interactions with host plants. Annu Rev Ecol Syst 29:319-343. https: //doi.org/10.1146/annurev.ecolsys.29.1.319

Sanghera GS, Wani SH, Wasim H, Singh NB (2011) Engineering cold stress tolerance in crop plants. Curr Genomics 12:30-43. http://www.ncbi.nlm.nih.gov/pmc/articles/ PMC 3129041

Scebba F, Sebastiani L, Vitagliano C (1998) Changes in activity of antioxidative enzymes in wheat (*Triticum aestivum*) seedlings under cold acclimation. Physiol Plantarum 104:747-752. https://doi.org/10.1034/j.1399-3054.1998. 1040433.x

Schardl C L, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal wendophytes. Annu Rev of Plant Biol 55:315-340. https://doi.org/10.1146/annurev. arplant.55.031903.141735

Shao GC, Lan JJ, Yu SE, Liu N, Guo RQ, She DL (2013) Photosynthesis and growth of winter wheat in response to waterlogging at different growth stages. Photosynthetica 51:429-437. https:// doi.org/10.1007/s11099-013-0039-9

Shen WY, Nada K, Tachibana S (2000) Involvement of polyamines in the chilling tolerance of cucumber cultivars. Plant Physiol 124:431-439. https://doi.org/10.1104/pp.124.1.431

Shimono H, Okada M, Kanda E, Arakawa I (2007) Low temperature-induced sterility in rice: Evidence for the effects of temperature before panicle initiation. Field Crops Res 101:221-231. https://doi.org/10.1016/j.fcr.2006.11.010

Siegel MR, Latch GCM, Johnson MC (1990) Fungal endophytes of grasses. Annu Rev Ecol Syst 21:275-297.

Smith D (1951) Root branching of alfalfa varieties and strains6. Agron J 43:573-573.

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

Svačina P, Středa T, Chloupek O (2014) Uncommon selection by root system size increases barley yield. Agron Sustain Dev 34: 545-551. https://doi.org/10.1007/s13593-013-016 0-y

Urbatzka P, Graß R, Haase T, Schüler C, Heß J (2012) Influence of different sowing dates of winter pea genotypes on winter hardiness and productivity as either winter catch crop or seed legume. Eur J Agron 40:112-119. https://doi.org/10. 1016/ j.eja.2012.03.001

Vestena S, Cambraia J, Ribeiro C, Oliveira JA, Oliva MA (2011) Cadmium-induced oxidative stress and antioxidative enzyme response in water hyacinth and salvinia. Brazilian J Plant Physiol 23:131-139. http://dx.doi.org/10.1590/S1677-04202011000200005

Viswanathan C, Zhu JH, Zhu JK, (2007) Cold stress regulation of gene expression in plants. Trends in Plant Sci 12**:**444-451. https://doi.org/10.1016/j.tplants.2007.07.002

Wang HF, He GX, Wang JH, Dong YY (2012) Monitoring winter wheat freeze injury based on multi-temporal data. Intell Autom Soft Co 18:1035-1042. https://doi.org/10.1080/10798587. 2008.10643308

Wheatley WM, Kemp HW, Simpson WR, Hume DE, Nicol HI, Kemp DR, Launders, TE (2007) Viability of endemic endophyte (*Neotyphodium lolii*) and perennial ryegrass (*Lolium perenne*) seed at retail and wholesale outlets in south-eastern Australia. Seed Sci Technol 35:360-370. https://doi.org/10.15258/sst.2007.35.2.11

White JF, Sullivan RF, Moy M, Meyer W, Cabral D (2001) Evolution of *Epichloë/ Neotyphodium* endophytes and other clavicipitalean biotrophs. Symbiosis: Mechanisms and Model Systems (Cellular Origin, Life in Extreme Habitats and Astrobiology). https://doi.org/10. 1007/0-306-48173-1_26

Xin Z, Browse J (2010) Cold comfort farm: the acclimation of plants to freezing temperatures. Plant Cell Environ 23:893-902. https://doi.org/10.1046/j.1365-3040.2000.00611.x

Yarzábal LA (2014) Cold-tolerant phosphate-solubilizing microorganisms and agriculture development in mountainous regions of the world.113-137. https://doi.org/10.1007/978-3-319-08216-5_5

Young CA, Hume DE, Mcculley RL (2013) Forages and pastures aymposium: fungal endophytes of tall fescue and perennial ryegrass: Pasture friend or foe? J Anim Sci 91:2379-2394. https://doi.org/10.2527/jas.2012-5951

Yves C, RéAl M, Paul N, Annick B (2009) An indoor screening method for improvement of freezing tolerance in alfalfa. Crop Sci 49:809-818. http://dx.doi.org/10.2135/cropsci2008.09.0539

Zhang DF, Li FQ, Bing, JM (2000) Eco-environmental effects of the Qinghai-Tibet Plateau uplift during the Quaternary in China. Environ Geol 39:1352-1358. https://doi.org/10.1007/s002540000174

Zhang T, Zhang YQ, Liu HY, Wei YZ, Li HL, Su J, Zhao LX, Yu LY (2013) Diversity and cold adaptation of culturable endophytic fungi from bryophytes in the Fildes Region, King George Island, maritime Antarctica. Fems Microbiol Lett 341: 52-61. https://doi.org/10.1111/1574-6968.12090

Zhang XQ, Huang GQ, Huang ZL, Bian XM, Jiang XH (2012) Effects of low temperature on freezing injury of various winter wheat cultivars at different sowing time. Agricultural Science and Technology: English Edition 13:2332-2337.

Zhang XX, Li CJ, Nan ZB (2010) Effects of cadmium stress on growth and anti-oxidative systems in *Achnatherum inebrians* symbiotic with *Neotyphodium gansuense*. J Hazard mater 175:703-709. https://doi.org/10.1016/j.jhazmat.2009.10.066

Zheng GW, Li LX, Li WQ (2016) Glycerolipidome responses to freezing- and chilling-induced injuries: examples in *Arabidopsis* and rice. Bmc Plant Biol 16:70-85. https://doi.org/10.1186/s12870-016-0758-8

Zhou LY, Li CJ, Zhang XX, Johnson R, Bao GS, Yao X, Chai Q (2015) Effects of cold shocked *Epichloë* infected *Festuca sinensis* on ergot alkaloid accumulation. Fungal Ecol 14:99-104. https://doi.org/10.1016/j.funeco.2014.12.006

Żurek M, Wiewióra B, Żurek G, Prończuk M (2012) Occurrence of endophyte fungi on grasses in Poland–Review. Fungal Ecol 5: 353-356. https://doi.org/10.1016/j.funeco.2011.07.007