Bangladesh J. Bot. **47**(3): 699-709, 2018 (September) Special

INTRA- AND INTERSPECIFIC COMPETITION OF *ELYMUS NUTANS* GRISEB. AND *FESTUCA SINENSIS* KENG. EX EB ALEXEEV. INFECTED BY *EPICHLOË* ENDOPHYTE

WEIHU LIN, XIAOXIAO WANG, JIANJUN WANG, CLEMENT NZABANITA, WENBO XU, LIN YANG, HONGFANG XI, PEI TIAN*, YANBING WANG, MIAOMIAO LI AND MEINING WANG

College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, 730020, PR China

Keywords: Elymus nutans, Festuca sinensism, Grassland, Agricultural ecosystem, Mixture sowing, Epichloë endophyte

Abstract

This experiment was conducted both in greenhouse and in field plot to investigate the growth performance of *Elymus natans* and *Festuca sinensis* in different mixture sowing ratios (*F. sinensis*/*E. natans*: 0 : 10, 2 : 8, 4 : 6, 6 : 4 and 8 : 2). *F. sinensis*/*E. natans* at 4: 6 ratio significantly increased the plant height, leaf width, root length, tiller number and dry weight under greenhouse condition. *E. nutans* along with endophyte and *F. sinensis* showed significantly higher growth performance than *E. nutans* monocultures (0 : 10) in the greenhouse. Under field condition the growth parameters of *F. sinensis* at 4:6 ratio was also significantly higher than the other ratios. The study indicated that 4 : 6 ratio was the best combination for growth of *E. nutans* and *F. sinensis.* The study also showed that the presence of *Epichloë* endophyte in *F. sinensis* enhances the growth of this grass and neighbouring *E. natans* plants. This finding provides a theoretical basis for cultivating the *E. nutans* and *F. sinensis* with growth advantages.

Introduction

Grassland and agricultural ecosystems comprise over a wide range of grass species, and many species are interrelated. Plants and microbes, plants and animals, animals and microbes have intricate relationships (Nan and Li 2004). The mixture sowing of different grass species can affect grassland ecosystems through complementation of different niches (Zhang *et al.* 2008). Previous studies revealed that mixed pasture can increase grass yield and plays a pivotal role in community stability (Ma *et al.* 2007, Shi *et al.* 2007). It is a reality that mixture grassland ecosystem had an advantage over monoculture grassland ecosystem such as higher grass yield (Isbell *et al.* 2009). The advantage of a mixture has often been assimilated to a higher yield of the mixture when compared with an equal area divided between monocultures of the components in the same proportion as they occur in the mixture (Malézieux *et al.* 2009). In addition, increased biomass and resource acquisition of species mixtures relative to monocultures can result from facilitative interactions (Brooker *et al.* 2008, Schipanski and Drinkwater 2012). Therefore, mixture sowing is an important method to restore the diversity of grassland ecological species.

Festuca sinensis is an important cool-season grass species grazed by cattle and sheep and is of increasing importance in managed pastures, especially in cool and semi-arid regions of China (Zhou *et al.* 2015). The development and application of *F. sinensis* play important role in the establishment of grassland and restoration of degradation ecosystems (Tian *et al.* 2015). Its advantages include resistance to cold and to stress caused by saline and alkaline soils, successful over wintering capabilities, a broad adaptability to altitudes of 2000-4200 m, good agronomic performance and high yields (Zhou *et al.* 2015, Tian *et al.* 2018). *F. sinensis* is commonly lived

*Author for correspondence: <tianp@lzu.edu.cn>.

close to *Festuca ovina*, *Elymus nutans* and *Roegneria nutans*. In particular, the *F. sinensis* + *E. nutans* mixture sowing can increase its aboveground biomass (Wang *et al.* 2007). The mixed growing relationship is an important factor in the natural grassland ecosystem stability (Shi 2002, Wang 2013).

E. nutans is the valuable forage grass in the alpine regions and has the characters of resistance to cold, drought and pest that can be used to improve cereal crops, which play important role in the restoration of the disturbed grasslands and construction of artificial grasslands, as well as animal production (Chen *et al.* 2009). It grows in the grassland of north, northwest and southwest China particularly on Qinghai-Tibet Plateau with a broad adaptability to altitudes of 1000 - 5000 m (Lu 1993, Chen *et al.* 2009). This grass does not grow alone and always associated with *F. sinensis* and other grasses. The *F. sinensis* + *E. nutans* mixture sowing can increase its forage yield (Yang *et al.* 2010).

Most research showed that endophyte can increase the capability of anti-biotic and abiotic stresses of grasses. *Epichloë* endophytes increase the resistance of host grasses to insect (Brem and Leuchtmann 2001), drought stress (Malinowski and Belesky 2000), salt stress (Reza Sabzalian and Mirlohi 2010) and reduce the heavy metal effects (Zhang *et al.* 2014). The endophytes enhanced water use efficiency and retained the plants growth under limited water conditions through improvement of photosynthetic efficiency and promoting the absorption of nutrient (Xia *et al.* 2018). On natural grassland, most grasses are endophyte infected. *F. sinensis* is a perennial bunchgrass highly infected with the *Epichloë* endophyte (Nan and Li 2004). However, *E. nutans* is rarely found to be infected by endophyte (Nan 1996a).

The mixture sowing of *F. sinensis* + *E. nutans* are widely concerned by experts and their studies demonstrated that mixture sowing cultivation of *F. sinensis* + *E. nutans* facilitates grassland establishment and restoration (Shi *et al.* 2009, Li *et al.* 2009, Zheng *et al.* 2009). Previous studies mostly focused on different species mixture sowing, while ignored research on the mixture sowing ratios. The present study was designed both in greenhouse and field with different mixture sowing ratios to explore the Intra- and interspecific competition of *E. nutans* and *F. sinensis* infected by *Epichloë* endophyte by measuring plant growth indexes.

Materials and Methods

The seeds of *Festuca sinensis* were obtained from the Yuzhong campus of Pastoral Agriculture Experimental Station of Lanzhou University, China (35°560’ N, 104°080’ E, altitude 1718 m). The seeds of *E. nutans* were obtained from the Bai Green International Grass Industry (Beijing) Co. Ltd., (http://140227.71ab.com/). The seeds were harvested from endophyte-infected (E+) and endophyte-free (E-) plants on 25 August 2015 and preserved at 4°C for further investigation. The endophytes in seeds were detected by microscopic examination with aniline blue staining (Nan 1996a, b).

Seedlings were sown on soil in tray and kept in a controlled-environment at greenhouse from 1 April 2016 to 1 August 2016 (4 months) maintaining 25 ± 2°C temperature, 42 ± 2% relative humidity and 14/10 hrs light and dark cycle. Eleven different ratios of plants including E + *F. sinensis* monocultures (0 : 10), E- *F. sinensis* monocultures (0 : 10), *E. nutans* monocultures (0 : 10), E+ *F. sinensis* /*E. nutans* (2 : 8) (the number of seeds E+ *F. sinensis*/*E. nutans* = 2 : 8), E + *F. sinensis*/*E. nutans* (4 : 6) mixtures, E + *F. sinensis*/*E.nutans* (6 : 4) mixtures, E + *F. sinensis*/*E.nutans* (8 : 2) mixtures, E - *F. sinensis*/*E. nutans* (2 : 8) mixtures, E- *F. sinensis*/*E. nutans* (4 : 6) mixtures, E- *F. sinensis*/*E. nutans* (6 : 4) mixtures and E- *F. sinensis*/*E. nutans* (8 : 2) were studied. Sterile sandy-loam soil (1 : 3 v/v) collected from Lanzhou University experimental field station was taken in seedlings culture tray (30 × 20 × 10 cm in size) and 300 seeds were sown in each tray with three replications for each treatment. Seedling trays were kept randomly in a greenhouse maintaining 25 ± 2°C, 42 ± 2% relative humidity and 14/10 hrs light and dark cycle.

Field experiments were performed from 1 April, 2016 to 1 August, 2016 (4 months) at the Lanzhou University at Yuzhong Pastoral Agriculture Experimental Station. Seven different combinations of plants were established, including E+ *F. sinensis* monocultures (0 : 10), E- *F. sinensis* monocultures (0 : 10), *E. nutans* monocultures (0 : 10), E+ *F. sinensis*/*E. nutans* (2 :8) (the number of seeds E+ *F. sinensis*/*E. nutans*=2:8), E+ *F. sinensis*/*E. nutans* (4 : 6) mixtures, E- *F. sinensis*/*E. nutans* (2 : 8) and E- *F. sinensis*/*E. nutans* (4 : 6). Three replications were maintained for each combination. After germination of seeds and seedling development excess seedlings were removed to ensure the exact ratio. The plot size was 0.8 m^2 (1 × 0.8 m), randomly distributed in field and plot to plot distance was 30 cm.

Five seedlings of both *F. sinensis* (E+/E-) and *E. nutans* were randomly selected and carefully removed from the experimental seedlings trays and plots (i.e. greenhouse and field), washed with distilled water and then dried using filter paper. Plant height, root length, tiller number, stem diameter and leaf width of each plant from each treatment were noted. The harvested plants were separated into two categories *i.e*. below and aboveground. Dry weight was obtained after freezing (LABCONCO Freeze Dry Systems-Model 77530 series, America) the tissues at -80°C for 72 hrs until a constant weight. The dry weight of aboveground and belowground was weighed separately for each treatment to determine the total dry weight of plants.

Data were analysed with SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) to assess the effects of the *F. sinensis* (E+/E-)/*E. nutans* ratio on endophyte on different growth parameters. One-way ANOVA was used to test the treatment differences. A repeated-measure ANOVA with Fisher's least significant difference (LSD) test was used to determine whether differences between means were statistically significant or not. Statistical significance was defined at the 95% confidence level. Values were expressed as mean ± standard error.

Results and Discussion

Mixed sowing and *Epichloë* endophytes had significant effects on plant height in greenhouse. The maximum plant height of E+ *F. sinensis* was observed at 4 : 6 ratio which was significantly higher than monocultures (0 : 10) and other ratios (Fig. 1A). In case of E- *F. sinensis* the maximum height was also at 4 : 6 ratio. The height of *E. nutans* mixed with E+ *F. sinensis* was highest at 4 : 6 ratio (Fig. 1B). Similarly, the height of *E. nutans* mixed with E- *F. sinensis* was peaked at 4 : 6 ratio which was significantly higher than the 8 : 2 ratio (Fig. 1B). *Epichloë* endophytes had significant effects on plant height.

In the field conditions, the maximum plant height of E + and E- *F. sinensis* was recorded at 4:6 ratio which was significantly higher than monocultures (0 : 10) and 2 : 8 ratio (Fig. 1C). The height of *E. nutans* mixed with E + *F. sinensis* was maximum at 4:6 ratio which was also significantly higher than monocultures (0 : 10) and 2 : 8 ratio. While, the height of *E. nutans* mixed with E- *F. sinensis* was non-significant among the different combinations (Fig. 1D). *Epichloë* endophytes showed significant effects on plant height. At the 4 : 6 ratio, the plant height of *E. nutans* mixed with E + *F. sinensis* was significantly higher than E- *F. sinensis* (Fig. 1D).

Mixture sowing and *Epichloë* endophytes had significant effects on the number of tillers in the greenhouse. The number of tillers of E + and E- *F. sinensis* was maximum at 4:6 ratio that was significantly higher than monocultures (0 : 10) and other ratios (Fig. 2A). The number of tillers of *E. nutans* mixed with E+ *F. sinensis* was highest at 4 : 6 ratio which was significantly higher than other ratios (Fig. 2B). The number of tillers of *E. nutans* mixed with E- *F. sinensis* was maximum at 4 : 6 and 6 : 4 ratios which were significantly higher than the 2 : 8 ratio (Fig. 2B). At 4 : 6 and 6 :

4 ratios, significantly higher tillers of E + *F. sinensis* than E- *F. sinensis* was observed (Fig. 2A). On the other hand, *E. nutans* mixed with E+ *F. sinensis* showed significantly higher tillers than E- *F. sinensis* at 4 : 6 and 2 : 8 ratios (Fig. 2B).

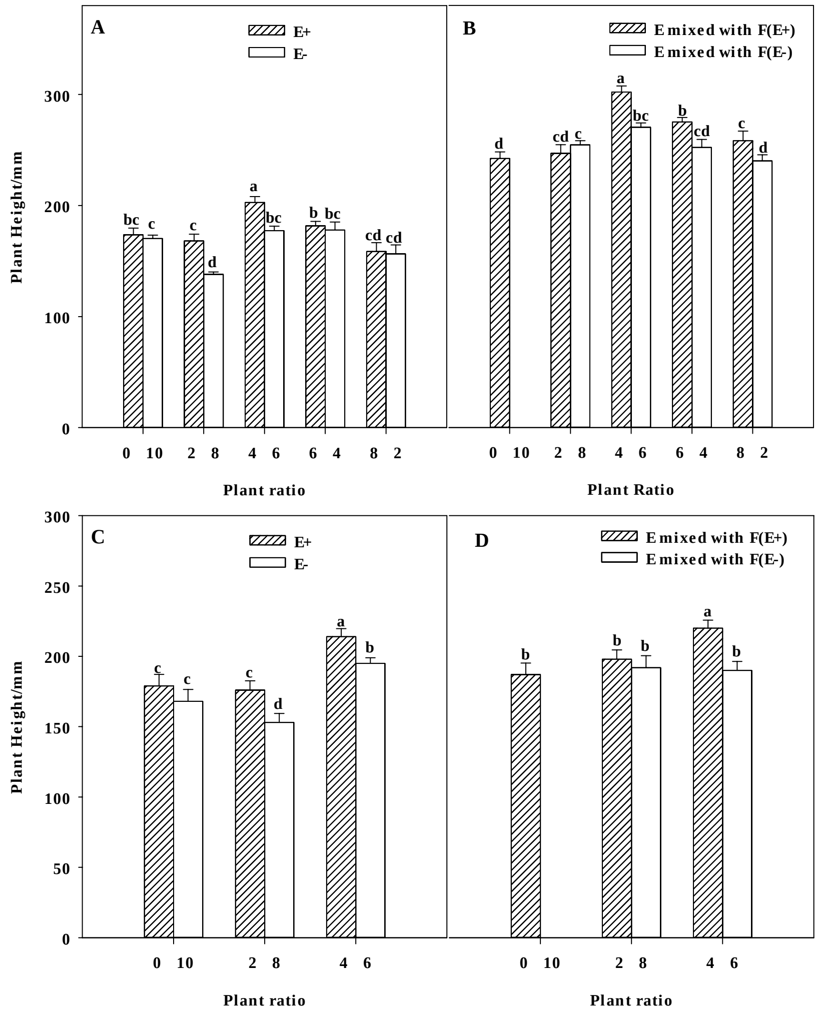

Fig. 1. Effects of mixture sowing and *F. sinensis–Epichloë* endophytes on the plant height of *F. sinensis* and *E. nutans.* Values are the mean ± standard error (SE), different lower-case letters indicates significant difference at $P < 0.05$. A: *F. sinensis* plant height under greenhouse, B: *E. nutans* plant height under greenhouse, C: *F. sinensis* plant height under field, D: *E. nutans* plant height under field.

In the field conditions, tillering in E + and E- *F. sinensis* was maximum at 4:6 ratio which was significantly higher than monocultures 0 : 10 and the 2 : 8 ratios (Fig. 2C). Besides the number of tillers of *E. nutans* mixed with E + *F. sinensis* was maximum at the 2 : 8 ratio which was significantly higher than monocultures 0 : 10 (Fig. 2D).

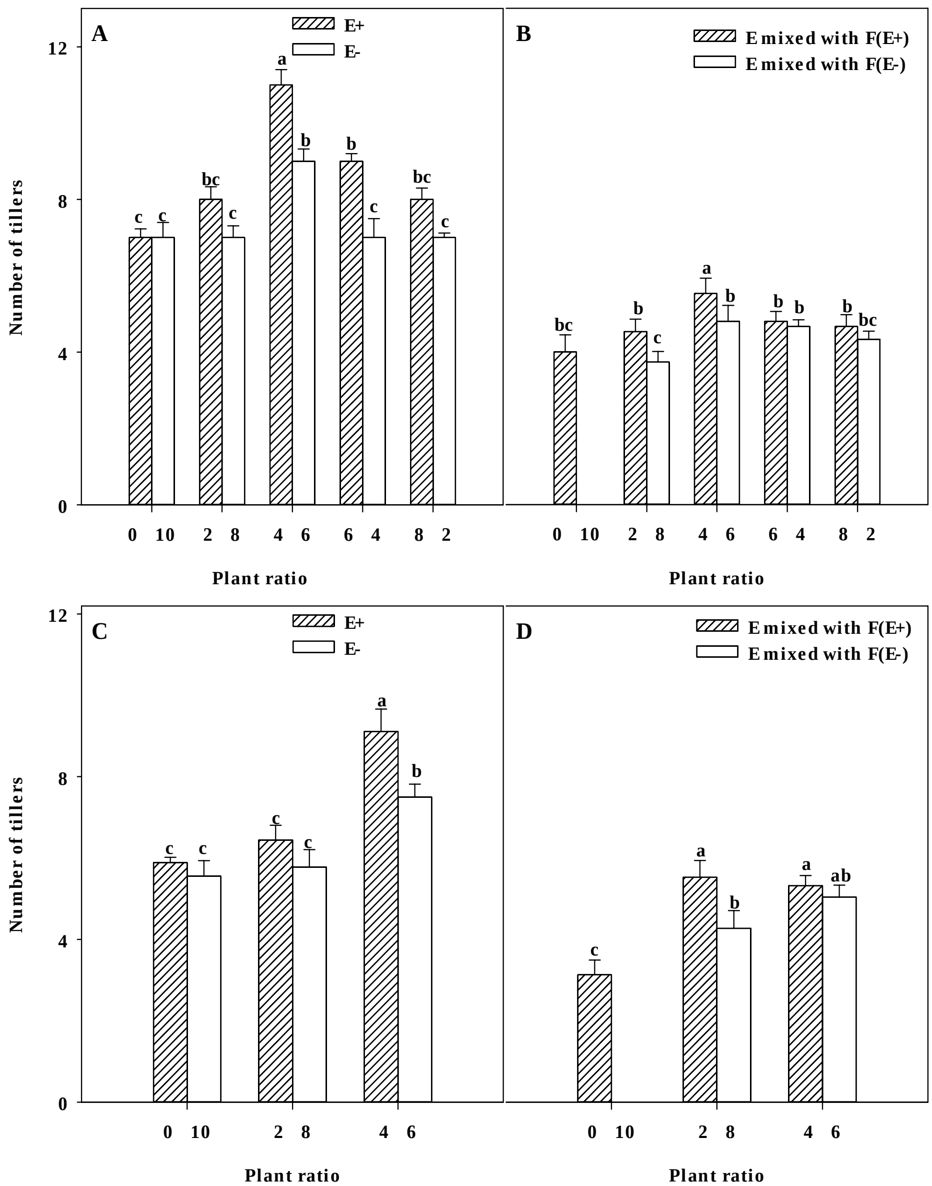

Fig. 2. Effects of mixture sowing and *F. sinensis–Epichloë* endophyte on the number of tillers of *F. sinensis* and *E. nutans.* Values are the mean ± standard error (SE), different lower-case letters indicates significant difference at $p < 0.05$. A: *F. sinensis* the number of tillers under greenhouse, B: *E. nutans* the number of tillers under greenhouse, C: *F. sinensis* the number of tillers under field, D: *E. nutans* the number of tillers under field.

Mixture sowing and *Epichloë* endophytes had significant effects on the stem diameter, leaf width and root length of both *F. sinensis* and *E. nutans* in the greenhouse. At 4 : 6 ratio, the stem diameter, leaf width and root length of E + *F. sinensis* was maximum which were significantly higher than monocultures (0 : 10) and other ratios (Table 1).

Table 1. The effects of mixture sowing and endophytes on stem diameter, leaf width and root length of *F. sinensis* and *E. nutans* under greenhouse.

Growth index	Plants	Ratios				
		0	2:8	4:6	6:4	8:2
Stem diameter (mm)	*E. nutans*	0.688 ± 0.024cd	-	-	-	-
	E+ *F. sinensis*	0.684 ± 0.022cd	0.724 ± 0.017cd	0.798 ± 0.022c	0.704 ± 0.017cd	0.659 ± 0.022cd
	E- *F. sinensis*	0.882 ± 0.020bc	0.676 ± 0.019cd	0.736 ± 0.023cd	0.697 ± 0.035cd	0.626 ± 0.039d
	E. nutans mixed with E+ *F. sinensis*	-	0.912 ± 0.031bc	1.022 ± 0.036a	0.990 ± 0.042a	0.960 ± 0.051ab
	E. nutans mixed with E- *F. sinensis*	-	0.826 ± 0.030bc	0.925 ± 0.035b	0.958 ± 0.025ab	0.944 ± 0.049ab
Leaf width (mm)	*E. nutans*	1.769 ± 0.090cd	-	-	-	-
	E+ *F. sinensis*	1.692 ± 0.032cd	1.737 ± 0.055cd	2.046 ± 0.085ab	1.804 ± 0.049cd	1.768 ± 0.046cd
	E- *F. sinensis*	1.888 ± 0.025c	1.581 ± 0.070d	1.969 ± 0.059b	1.710 ± 0.054cd	1.705 ± 0.063cd
	E. nutans mixed with E+ *F. sinensis*	-	1.942 ± 0.062bc	2.276 ± 0.047a	2.142 ± 0.129a	1.882 ± 0.055c
	E. nutans mixed with E- *F. sinensis*	-	1.812 ± 0.062cd	2.125 ± 0.067a	2.060 ± 0.131ab	1.802 ± 0.085cd
Root length (cm)	*E. nutans*	4.67 ± 0.17c	-	-	-	-
	E+ *F. sinensis*	4.62 ± 0.21c	4.49 ± 0.40c	5.44 ± 0.26ab	5.02 ± 0.24c	5.03 ± 0.15c
	E- *F. sinensis*	4.48 ± 0.20c	3.62 ± 0.13d	4.86 ± 0.64c	4.45 ± 0.13c	4.32 ± 0.17cd
	E. nutans mixed with E+ *F. sinensis*	-	4.91 ± 0.16c	5.82 ± 0.13a	4.91 ± 0.41c	4.40 ± 0.16cd
	E. nutans mixed with E- *F. sinensis*	-	4.82 ± 0.16c	5.94 ± 0.28a	5.32 ± 0.25bc	4.76 ± 0.18c

Values are the mean ± standard error (SE), with bars indicating different lower-case letters with significant differences between the different treatments at $p < 0.05$.

In the field conditions, the stem diameter of E + and E- *F. sinensis* was highest at 4 : 6 ratio which was significantly higher than monocultures (0 : 10). Mixture sowing had no significant effects on the stem diameter of *E. nutans* mixed with E + *F. sinensis* (Table 2).

Mixture sowing had also significant effects on the dry weight per seedling of both *F. sinensis* and *E. nutans* in the greenhouse. The dry weight of E+ and E- *F. sinensis* was significantly higher at 4 : 6 ratio than monocultures (0 : 10) (Table 3). In the field conditions, mixture sowing had no significant effects on the dry weight per seedling of E+ and E- *F. sinensis*. The dry weight of *E.*

nutans was maximum at 4 : 6 ratio. At 2 : 8 ratio, the dry weight of E + *F. sinensis* was significantly higher than E - *F. sinensis* at 4 : 6 ratio (Table 4).

Table 2. The effects of mixture sowing and endophytes on stem diameter, leaf width and root length of *F. sinensis* and *E. nutans* under field conditions.

Growth index	Plants	Ratios		
		0	2:8	4:6
Stem diameter (mm)	*E. nutans*	1.477 ± 0.084a	-	-
	E+ *F. sinensis*	1.021 ± 0.037c	1.158 ± 0.026bc	1.404 ± 0.050a
	E- *F. sinensis*	0.984 ± 0.062c	0.983 ± 0.067c	1.202 ± 0.038b
	E. nutans mixed with E+ *F. sinensis*	-	1.482 ± 0.100a	1.560 ± 0.076a
	E. nutans mixed with E- *F. sinensis*	-	1.211 ± 0.028b	1.442 ± 0.023a
Leaf width (mm)	*E. nutans*	4.741 ± 0.132b	-	-
	E+ *F. sinensis*	3.794 ± 0.135c	3.343 ± 0.155cd	4.281 ± 0.235bc
	E- *F. sinensis*	3.046 ± 0.190d	2.822 ± 0.084d	3.782 ± 0.126c
	E. nutans mixed with E+ *F. sinensis*	-	5.370 ± 0.198b	6.055 ± 0.201a
	E. nutans mixed with E- *F. sinensis*	-	5.183 ± 0.355b	5.612 ± 0.307ab
Root length (cm)	*E. nutans*	6.8 ± 0.2bc	-	-
	E+ *F. sinensis*	7.3 ± 0.3b	7.6 ± 0.3ab	8.3 ± 0.2a
	E- *F. sinensis*	7.0 ± 0.2bc	6.5 ± 0.3c	7.5 ± 0.3ab
	E. nutans mixed with E+ *F. sinensis*	-	8.2 ± 0.2a	8.3 ± 0.2a
	E. nutans mixed with E- *F. sinensis*	-	7.8 ± 0.2ab	7.7 ± 0.2ab

Values are the mean ± standard error (SE), with bars indicating different lower-case letters with significant differences between the different treatments at $p < 0.05$.

The key results of this study were that mixture sowing ratios had significant effects on *F. sinensis* and *E. nutans* growth performance. Especially at the 4 : 6 ratio, both plants showed excellent growth performance. The plant height, tiller numbers, stem diameter, leaf width, root length and dry weight of mixture sowing plants were also higher than monoculture. In addition, the present investigation showed that the presence of *Epichloë* endophyte in *F. sinensis* enhanced the growth of this grass and also neighbouring *E. natans* plants.

Previous research had indicated that inequalities in size within species, by the larger individuals, can result in the pre-emption of resources (Howard and Goldberg 2001). This means that larger individuals generally, had the competitive advantage over smaller individuals in mixture sowing combinations (Gu *et al.* 2009). The previous study proved that the competitive ability of *E. nutans* was stronger than *F. sinensis* under mixted sowing conditions (Gu *et al.* 2012). However, scientists rarely studied the effect of mixture sowing ratios on plant growth performance. In fact, different mixture sowing ratios significantly affect plant growth (Caballero *et al.* 1995, Lithourgidis *et al.* 2006). The present findings indicated that different growth performances of *F. sinensis* and *E. nutans* are different under different mixture sowing ratios. Mixture sowing ratios had significant effects on the plant heights and tiller numbers of *F. sinensis* and *E. nutans*. The plant height and tiller number of these two taxa increased in mixture sowing ratios in both field and greenhouse conditions particularly at 4 : 6 ratio which might be due to the difference in their niches. Changing the utilization of resources in the growth environment was supported by the findings of Caballero *et al*. (1995). In addition, plant height of *E. nutans* was higher than *F. sinensis* under the greenhouse condition, while the tiller number of *F. sinensis* was

Table 3. The effects of mixture sowing and endophytes on plant dry weight per seedling of *F. sinensis* and *E. nutans* under greenhouse conditions.

Growth index	Plants	Ratios				
		0	2:8	4:6	6:4	8:2
Dry weight (g)	*E. nutans*	0.0600 ± 0.0040b	-	-	-	-
	E+ *F. sinensis*	0.0555 ± 0.0053bc	0.0622 ± 0.0098ab	0.0800 ± 0.0047a	0.0655 ± 0.0050ab	0.0633 ± 0.0050ab
	E- *F. sinensis*	0.0411 ± 0.0045c	0.0466 ± 0.0033bc	0.0766 ± 0.0060a	0.0688 ± 0.0042ab	0.0644 ± 0.0037ab
	E. nutans mixed with E+ *F. sinensis*	-	0.0655 ± 0.0017ab	0.0744 ± 0.0037ab	0.0544 ± 0.0050bc	0.0500 ± 0.0033bc
	E. nutans mixed with E- *F. sinensis*	-	0.0588 ± 0.0020bc	0.0688 ± 0.0045ab	0.0611 ± 0.0035b	0.0555 ± 0.0037bc

Values are the mean ± standard error (SE), with bars indicating different lower-case letters with significant differences between the different treatments at $p < 0.05$.

Table 4. The effects of mixture sowing and endophytes on plant dry weight per seedling of *F. sinensis* and *E. nutans* under field conditions.

Growth index	Plants	Ratios		
		0	2:8	4:6
Dry weight (g)	*E. nutans*	0.5222 ± 0.0251d	-	-
	E+ *F. sinensis*	0.6522 ± 0.0405bc	0.7088 ± 0.0170ab	0.7388 ± 0.0406ab
	E- *F. sinensis*	0.5777 ± 0.0236cd	0.5866 ± 0.0345cd	0.6233 ± 0.0302bc
	E. nutans mixed with E+ *F. sinensis*	-	0.6077 ± 0.0263c	0.7755 ± 0.0267a
	E. nutans mixed with E- *F. sinensis*	-	0.6211 ± 0.0272bc	0.6833 ± 0.0284b

Values are the mean ± standard error (SE), with bars indicating different lower-case letters with significant differences between the different treatments at $p < 0.05$.

more than *E. nutans* under both the greenhouse and field conditions. This phenomenon might be considered as an adaptive of plants performance, to increase its competitiveness by increasing plant height and tiller numbers. Significantly increased leaf width, root length and dry weight of *F. sinensis* and *E. nutans* in mixture sowing particularly at 4 : 6 ratio under the greenhouse and field conditions compared with the monoculture are similar to the findings reported by Chen *et al.* (2011). This study also demonstrated that mixture sowing promotes both the mixed plant's growth performance.

Previous research had indicated that Endophyte infection, under natural conditions, can increase the height of plants, tiller numbers and biomass of host grasses in greenhouse and field conditions (Zhang *et al.* 2015) which supports the present observations. The results demonstrated that the presence of *Epichloë* endophyte in *F. sinensis* enhanced the growth of this grass and also neighbouring *E. natans* plants. This might be because *Epichloë* endophyte in *F. sinensis* enhanced plants competitiveness (Li *et al.* 2009). Earlier studies with E+ and E- in *F. sinensis* had revealed that *Epichloë* endophytes promote the germination ability and growth of seedling under drought and cold stress (Peng *et al.* 2013, Wang *et al.* 2017). In the present study, the root length of E+ *F. sinensis* has a significant level of higher - than E- *F. sinensis* under the 2:8 ratio. Consequently, mixture sowing and *Epichloë* endophyte and these two factors impact on plant performance were significant. These results demonstrated that mixture sowing is reasonable to promote plant growth it is in agreement with the results reported by Caballero *et al*. (1995).

It may be concluded that the mixture sowing plants increase its competitiveness by increasing plant height and tiller numbers and *Epichloë* endophyte in *F. sinensis* enhances the plant height, tiller numbers, stem diameter, leaf width and root length of this grass and also neighbouring *E. natans* plants under the mixture sowing conditions. The study provides a theoretical basis for cultivating the *F. sinensis* and *E. nutans* with growth advantages.

Acknowledgements

This work was supported by the Natural Science Foundation of China (31502001) and the National Basic Research Program of China (2014CB138702).

References

Brem D and Leuchtmann A 2001. *Epichloë* grass endophytes increase herbivore resistance in the woodland grass *Brachypodium sylvaticum*. Oecologia **126**: 522-530.

Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, Liancourt P, TielbÖRger K, Travis JMJ, Anthelme F, Armas C, Coll L, Corcket E, Delzon S, Forey E, Kikvidze Z, Olofsson J, Pugnaire F, Quiroz CL, Saccone P, Schiffers K, Seifan M, Touzard B and Michalet R 2008. Facilitation in plant communities: the past, the present, and the future. Journal of Ecology **96**: 18-34.

Caballero R, Goicoechea EL and Hernaiz PJ 1995. Forage yields and quality of common vetch and oat sown at varying seeding ratios and seeding rates of vetch. Field Crops Research **41**: 135-140.

Chen SY, Ma X, Zhang XQ and Chen ZH 2009. Genetic variation and geographical divergence in *Elymus nutans* Griseb. (Poaceae: Triticeae) from West China. Biochemical Systematics and Ecology **37**: 716-722.

Chen Z, Tian F, Wu G and Niu J 2011. Effects of overseeding grass on aboveground biomass of different economic group in Maqu alpine desertified meadow pasture. Chinese Journal of Grassland **33**: 58-62.

Gu MH, Dong SK and Du GZ 2009. Interspecific interactions in binary mixtures of *Elymus nutans, Festuca sinensis* and *Festuca ovina* on the eastern Qinghai-Tibetan plateau. New Zealand Journal of Agricultural Research **52**: 171-177.

Gu MH, Wang T and Xie ZK 2012. Competitive hierarchy among three perennial grasses under clipping and fertilization through three growing years on the eastern Qinghai-Tibetan Plateau. Procedia Environmental Sciences **13**: 151-157.

Howard TG and Goldberg DE 2001. Competitive response hierarchies for germination, growth, and survival and their influence. Ecology **82**: 979-990.

Isbell FI, Wayne PH and Wilsey BJ 2009. Species interaction mechanisms maintain grassland plant species diversity. Ecology **90**: 1821-1830.

Li A, Gu M, Zhang S, Zhou X, Wang Y, Li W and Du G 2009. Effects of species richness on weed invasion in an artificial grassland ecosystem in eastem Tibetan Plateau. Chinese Journal of Ecology **28**: 177-181.

Lithourgidis AS, Vasilakoglou IB, Dhima KV, Dordas CA and Yiakoulaki MD 2006. Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. Field Crops Research **99**: 106-113.

Lu BR 1993. Meiotic studies of *Elymus nutans* and *E. jacquemontii* (*Poaceae, Triticeae*) and their hybrids with *Pseudoroegneria spicata* and seventeen *Elymus* species. Plant Systematics and Evolution **186**: 193-212.

Ma Y, Shang Z, Shi J, Dong Q and Long R 2007. Studies on allocate skills of artificial community of "Black Soil Type" degraded grassland in the Yellow River source region. Acta Agriculturae Boreali Occidentalis Sinica **16**: 1-6.

Malézieux E, Crozat Y, Dupraz C, Laurans M, Makowski D, Ozier-Lafontaine H, Rapidel B, Tourdonnet S and Valantin-Morison M 2009. Mixing plant species in cropping systems: concepts, tools and models. A review. Agronomy for Sustainable Development **29**: 43-62.

Malinowski DP and Belesky DP 2000. Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Science **40**: 923-940.

Nan ZB 1996a. Incidence and distribution of endophytic fungi in seeds of some native and introduced grasses in China. Acta Prataculturae Sinica **5**: 1-8.

Nan ZB 1996b. Incidence and distribution of endophytic fungi in seedlings and plants of some native and introduced grasses in China. Acta Prataculturae Sinica **5**: 13-17.

Nan ZB and Li CJ 2004. Roles of the grass-*Neotyphodium* association in pastoral agriculture systems. Acta Ecologica Sinica **24**: 605-616.

Peng QQ, Li CJ, Song ML and Nan ZB 2013. Effects of seed hydropriming on growth of *Festuca sinensis* infected with *Neotyphodium* endophyte. Fungal Ecology **6**: 83-91.

Reza Sabzalian M and Mirlohi A 2010. *Neotyphodium* endophytes trigger salt resistance in tall and meadow fescues. Journal of Plant Nutrition and Soil Science **173**: 952-957.

Schipanski ME and Drinkwater LE 2012. Nitrogen fixation in annual and perennial legume-grass mixtures across a fertility gradient. Plant Soil **357**: 147-159.

Shi J 2002. Study on grass instoduction and technology of mixed seeding in alpine-cold pasture. Chinese Qinghai Journal of Animal and Veterinary Sciences **32**: 5-7.

Shi J, Hong F, Ma Y, Zhang D, Wang Y, Li R and Yang S 2009. Artificial control impact on community character of gramineous mixed grassland. Acta Agrestia Sinica **17**: 745-751.

Shi J, Ma Y, Dong Q, Wang Y and Wang L 2007. The selection experiment of fine forages in 'Black Soil Type' degraded grasslands. Acta Agrestia Sinica **15**: 543-555.

Tian P, Kuang Y, Lin WH, Wang JJ and Nan ZB 2018. Shoot morphology and alkaloid content of *Epichloë* endophyte–*Festuca sinensis* associations. Crop Pasture Science **69**: 430-438.

Tian P, Kuang Y and Nan ZB 2015. The characteristics of *Festuca sinensis* and its breeding potential. Pratacultural Science **32**: 1079-1087.

Wang JJ, Zhou YP., Lin, WH, Li MM, Wang MN, Wang ZG, Kuang Y and Tian P 2017. Effect of an *Epichloë* endophyte on adaptability to water stress in *Festuca sinensis*. Fungal Ecology **30**: 39-47.

Wang KY 2013. Effect of three sodium salt stress on seed germination of *Festuca sinensis*. Guizhou Agricultural Sciences **41**: 108-111.

Wang L, Bi Y, Ma Y and Shi J 2007. Study of the optimal combination of mixed seeding artificial pasture in "Black soil type" degraded meadow. Journal of Qinghai University (Nature Science) **25**: 1-5.

Xia C, Christensen MJ, Zhang X and Nan Z 2018. Effect of *Epichloë* gansuensis endophyte and transgenerational effects on the water use efficiency, nutrient and biomass accumulation of *Achnatherum inebrians* under soil water deficit. Plant Soil. 1-17.

Yang H, Ma Y and Shi J 2010. Study on interspecific competition of mixed artificial grassland in "Black soil beach". Chinese Qinghai Journal of Animal and Veterinary Sciences **40**: 1-3.

Zhang H, Yang H, Li C, Liu J, Ge X and Zhang L 2008. Research on the dynamics of productive forces of Alfalfa+awnless brome man-made grassland under the different patterns of mixed sowing. Journal of Inner Mongolia University for Nationalities **23**: 55-58.

Zhang XX, Nan ZB and Li CJ 2014. Research progress of improved resistance of the grass to the heavy metal stress by endophyte. Pratacultural Science **31**: 1466-1474.

Zhang XX, Xia C and Nan ZB 2015. Effects of symbiotic *Epichloë* gansuensis endophyte on drunken horse grass (*Achnatherum inebrians*) growth and seed production. New Zealand Journal of Agricultural Research **58**: 234-240.

Zheng H, Chen Z, Niu J and Gao Y 2009. Effects of interseeding on plant diversity and productivity in Maqu alpine desertified meadow. Acta Prataculturae Sinica **18**: 28-33.

Zhou LY, Zhang XX, Li CJ, Christensen MJ and Nan ZB 2015. Antifungal activity and phytochemical investigation of the asexual endophyte of *Epichloë* sp. from *Festuca sinensis*. Science China-Life Sciences **58**: 821-826.

(Manuscript received on 25 July, 2018; revised on 4 September, 2018)