Comprehensive evaluation of endophytic fungi and rhizosphere soil fungi on the growth of *Achnatherum inebrians*

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Abstract. This study was conducted to clarify the effect of endophytic fungi and rhizosphere soil fungi on the growth of *Achnatherum inebrians*. In this study, the seeds of *A. inebrians* with endophyte-infected (EI) and endophyte-free (EF) were used as materials. Eight fungi isolated from rhizosphere soil were inoculated through germination and greenhouse pot experiment. The results showed that the endophytes, rhizosphere soil fungi and their combined effect all had significant effect on the seed germination and plant growth of *A. inebrians*, and the affected factors varied with the tested materials and strains. Through comprehensive evaluation of principal component analysis and subordinate function, it was found that the overall growth performance of EI was better than that of EF plants, and the strains that inhibited the growth of *A. inebrians* were *Cladosporium. sp*2 and *Fusarium sp*1.

Introduction

Grass endophytes often form reciprocal symbionts with most hosts, and some studies have shown that endophytes can improve resistance to biological and abiotic stresses of host. *Achnatherum inebrians* was a widespread bunch grass perennial herb of arid and semiarid harsh conditions in northwest China. Our previous research on *A.inebrians* found that the existence of endophytes significantly reduced the community diversity of root-associated fungi under field cultivation conditions (Zhong et al., 2018), while it significantly increased the diversity of the rhizosphere soil bacterial community (Ju et al., 2020). Under drought conditions, root-associated AMF diversity increased, but the diversity of root-related AMF decreased with increasing water (Zhong et al., 2021). The purpose of this study was to research: (1) the effect of endophyte on the effect of rhizosphere soil fungi; (2) the combined effect between endophyte and rhizosphere soil fungi for the growth of *A. inebrians*.

Materials and methods

A total of 8 fungal strains derived from the rhizosphere soil of *A. inebrians* growing naturally in the field were used in this study: *Cladosporium sp*1 (F1), *Cladosporium. sp*2 (F2), *Clonostachys sp* (F3), *Fusarium sp*1 (F4), *Fusarium sp*2 (F5), *Fusarium sp*3 (F6), *Mortierella sp* (F7) and *Sarocladium sp* (F8). To the germination experiment, the disinfected seeds of *A.inebrians*, with 75% alcohol for 3 min, 1% NaClO for 5 min and sterile water for 4 times, were immersed 24 h in the prepared spore suspension (1×10^8 spores/ml), each petri dish contained 50

seeds for 4 replicates, the untreated seeds were taken as the control, the germination indictors were calculated after end the experiment. To the greenhouse pot experiment, each pot contained 200 grams of high pressure sterilized culture substrate (121℃ for 2 h). Each treatment was replicated 4 times and each replicate contained 9 plants. The greenhouse culture conditions were 27℃/23℃(day/night), 16/8 h (light/dark) and RH 55±10%. The cultured strains (steriled PDA liquid medium, OD=1.0) were inoculated on the 60th day of the growth of *A. inebrians*. The plant height, root length and biomass indictors were measured after 1 month. Comprehensive evaluation was executed using the principal component analysis method and the subordinate function method (Cao et al., 2015) according to measured indictors.

Results and Discussion

The results of germination and greenhouse pot experiment showed that the endophytes, rhizosphere soil fungi, and the combination of both had a significant (P<0.05) effect on seed germination and greenhouse growth indictors (Figure 1, Figure 2). For example, the endophytes had a significant (P<0.05) effect on seedling fresh weight and seedlings dry weight of germination indictors (Figure 1C). In greenhouse condition, endophytes and rhizosphere fungi all had significant (P<0.05) effect on plant height, root length, shoot and root biomass (Figure 2). In order to reduce the interference between data and improve the accuracy of data analysis. In this study, all single indicators were converted into a smaller number and more effective principal component indicators by PCA analysis. The cumulative contributions from first to third of principal component reached 85.9% in EI and 81.9% in EF plants (Table 1), which were enough to represent a large part of the information of the original indicators. At present, subordinate function analysis is one common method used in the evaluation of abiotic resistance. Based on PCA results, we calculated comprehensive evaluation value (D) of each treatment. Furthermore, the EI and EF *A. inebrians* tolerance to rhisophere fungi were ranked according to the D value (Table 2). The results indicated that F2 and F4 had the lower D value of the tested treatments.

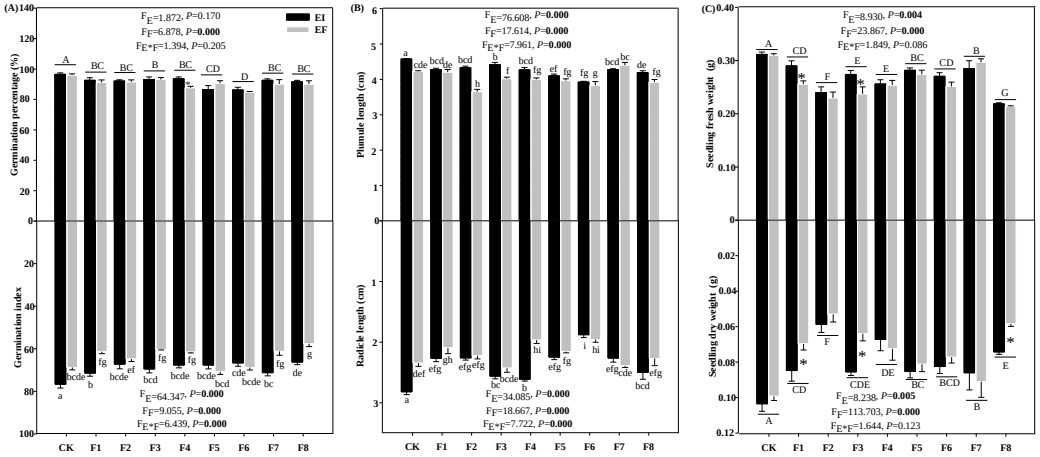

Figure 1 Two-way ANOVA for the effects of endophyte (E) and rhizosphere soil fungi (F) on germination indicators of *A.inebrian.*

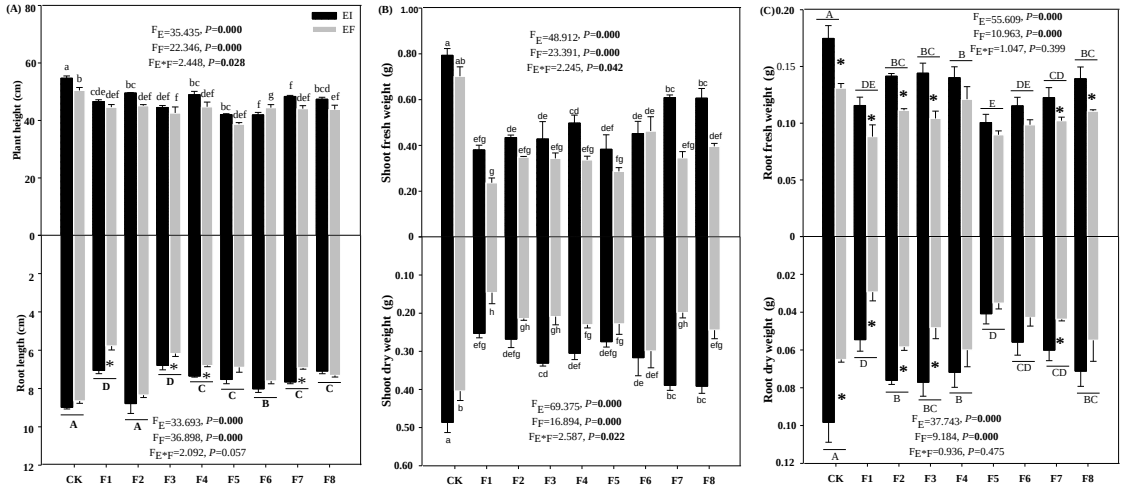

Figure 2 Two-way ANOVA for the effects of endophyte (E) and rhizosphere soil fungi (F) on greenhouse growth indicators of *A. inebrians*

Table 1 Eigenvalue, contributions and loading factor of each component.

Items	Traits	Principal components					
		EI			EF		
		1	2	3	1	2	3
Eigenvalue		7.015	2.123	1.166	5.193	2.620	2.012
Contributive ratio (%)		58.460	17.690	9.722	43.271	21.829	16.769
Cumulative contribution (%)		58.460	76.150	85.872	43.271	65.099	81.868
Loading factor of each component	Germination percentage	0.120	-0.111	-0.268	0.089	0.083	0.286
	Germination index	0.111	0.238	-0.093	0.068	0.068	-0.376
	Plumule length	0.121	-0.047	-0.280	0.041	0.313	0.158
	Radicle length	0.112	-0.105	-0.319	0.056	0.094	0.352
	Seedling fresh weight	0.050	0.390	-0.106	0.107	0.278	-0.088
	Seedling dry weight	0.063	0.379	-0.049	0.111	0.267	-0.129
	Plant height	0.123	-0.108	0.089	0.147	-0.045	0.083
	Root length	0.062	0.012	0.618	0.143	-0.151	-0.115
	Shoot fresh weight	0.115	0.026	0.288	0.175	-0.029	-0.048
	Shoot dry weight	0.109	0.073	0.233	0.166	-0.040	-0.142
	Root fresh weight	0.123	-0.151	0.044	0.150	-0.143	0.119
	Root dry weight	0.122	-0.158	0.060	0.131	-0.211	0.122

Table 2 The standardized scores values (FAC), comprehensive scores values (F), subordinative function value (U), comprehensive evaluation value (D), weights and rank.

Treatment		Standardized scores value			Comprehensive scores values			Subordinative function value			Comprehensive evaluation value (D)	Rank
		FAC1	FAC2	FAC3	F1	F2	F3	U1	U2	U3		
EI	CK	2.251	0.800	0.645	6.020	1.178	0.703	0.980	0.913	0.706	4.736	1
	F1	-0.364	0.691	-1.254	-0.974	1.018	-1.368	0.216	0.870	0.084	1.949	5
	F2	-0.102	-1.484	0.866	-0.274	-2.184	0.945	0.292	0.000	0.779	1.631	7
	F3	0.169	-0.085	-1.510	0.453	-0.125	-1.647	0.372	0.559	0.000	1.818	6
	F4	0.073	-1.054	-0.612	0.195	-1.551	-0.668	0.344	0.172	0.294	1.493	9
	F5	-1.103	0.968	-0.049	-2.950	1.424	-0.053	0.000	0.980	0.479	1.965	4
	F6	-1.088	0.664	1.481	-2.911	0.977	1.616	0.004	0.859	0.980	2.345	3
	F7	0.200	0.683	0.234	0.536	1.006	0.255	0.381	0.866	0.571	2.917	2
	F8	-0.036	-1.184	0.198	-0.096	-1.743	0.216	0.312	0.120	0.560	1.621	8
	Weight value							0.667	0.202	0.111		
EF	CK	2.427	0.460	0.093	5.587	0.752	0.134	0.980	0.657	0.619	4.217	1
	F1	-1.077	0.900	0.270	-2.479	1.471	0.387	0.000	0.804	0.674	2.281	4
	F2	0.002	-1.499	0.154	0.004	-2.451	0.220	0.302	0.000	0.638	1.609	8
	F3	-0.445	0.003	1.250	-1.024	0.005	1.791	0.177	0.504	0.980	2.635	3
	F4	-0.059	-0.624	-0.201	-0.135	-1.020	-0.288	0.285	0.293	0.527	1.891	7
	F5	-0.611	0.830	-1.139	-1.407	1.356	-1.632	0.130	0.781	0.235	1.913	6
	F6	-0.047	-0.432	-1.890	-0.107	-0.707	-2.709	0.288	0.358	0.000	1.248	9
	F7	0.042	1.423	0.602	0.096	2.327	0.862	0.313	0.980	0.778	3.437	2
	F8	-0.233	-1.060	0.862	-0.536	-1.733	1.236	0.236	0.147	0.859	2.015	5
	Weight value							0.518	0.261	0.201		

Conclusions

The endophytes, rhizosphere soil fungi and their combination had significant effect on seed germination and greenhouse growth indictors of *A. inebrians*, especially for germination index, plumule length, radicle length, root length, shoot fresh weight and shoot dry weight. The overall growth performance of EI plants was brtter than EF, and a strain of *Cladosporium sp* and of *Fusarium sp* largely inhibited *A. inebrians* growth.

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