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Isolation and species diversity of arbuscular mycorrhizal fungi in the rhizosphere of *Puccinellia tenuiflora* of Songnen saline-alkaline grassland, China

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Key words: Morphological identification, Spore density, Species richness, Diversity indexes, Colonization intensity

Abstract: Salinization has led to the deterioration of the ecological environment, affected the growth of plants, and hindered the development of agriculture and forestry. Arbuscular mycorrhizal (AM) fungi, as important soil microorganisms, play significant physiological and ecological roles in promoting plant nutrient absorption and improving soil structure. *Puccinellia tenuiflora* (Turcz.) Scribn. et Merr. in Songnen saline-alkaline grassland was selected as the research object to observe AM fungal colonization of the roots and explore the species and diversity of AM fungi in symbiotic association with *P. tenuiflora*. This study showed that AM fungi colonized in *P. tenuiflora* roots and formed a typical *Arum*–type mycorrhizal structure. A significant correlation was observed between vesicular abundance and the colonization intensity of mycorrhiza. Isolation and identification revealed 40 species of AM fungi in the rhizosphere of *P. tenuiflora*, belonging to 14 genera, of which two species could not be identified. The richness of the genus *Glomus* was the highest, accounting for 30% of the total species. *Funneliformis mosseae* and *Rhizophagus intraradices* were isolated from all the samples and were the species with the widest distribution in the rhizosphere of *P. tenuiflora*. Correlation analysis showed that pH only had a significant impact on the distribution of a few species, such as *Glomus pustulatum*, *Diversispora spurca*, *Glomus aggregatum*, *Rhizophagus clarum*, and *Acaulospora foveata*. The present study provides a theoretical basis for further exploring the resources of AM fungi in saline-alkaline soil.

Introduction

Songnen Plain is the primary distribution area of soda saline-alkali soil in China and also one of the three soda saline soil distribution areas in the world (Zhang and Feng, 2009; Wang *et al*., 2018), with a saline-alkali land area of 3.93×10^6 hm^2 (Li and Zhang, 2005). More seriously, due to the rapid development of agriculture, animal husbandry, overexploitation, and utilization of resources, as well as the increasingly arid climate, the salinized land in Songnen Plain have increased by about 1.7% every year since the second half of the 20th century (Lin *et al*., 1999). To make full use of the resources of saline-alkali land and for the sustainable development of the ecological environment, several measures for improving soil physical, chemical, biological, agronomic, and hydraulic engineering have been undertaken and have provided good results (Zhang *et al*., 2002). Among them, the application of arbuscular mycorrhizal (AM) fungi as a "biological fertilizer" to the saline-alkali land is of great help to improve plant tolerance and improve soil structure; it is considered to be a green and efficient method and plays an irreplaceable role in the ecosystem (Zhang *et al*., 2019; Deng *et al*., 2019).

AM fungi are one of the largest biomass components of soil microbial community (Wang *et al*., 2015); about 80% of bryophytes, pteridophytes, and spermatophytes, can be colonized by AM fungi to form a symbiotic system (Brundrett, 2009). This mycorrhizal symbiotic system has become a new type of bioremediation to cope with global change, which can shorten the restoration cycle of damaged and degraded ecosystems, improve the success rate of restoration and ensure the stability of restoration effects (van der Heijden *et al*., 2008). AM fungi play an important role in the remediation of saline soil in the following aspects: (1) improve the quality of saline-alkali soil by improving the physical and chemical properties of soil (Rilling *et al*., 2002), (2) significantly promote the absorption and utilization of water, mineral elements, and phosphorus to promote plant growth (Metwally and Abdelhameed, 2018; Parvin *et al*., 2020), (3) operate nutrients between mycorrhizal fungi and

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plants, mycorrhizal fungi and microorganisms, and plants and microorganisms through their huge mycelium network, to form a complete biological community (Yang *et al.*, 2015), etc.

Abundant AM fungi resources exist naturally in saline habitats of terrestrial ecosystems; for example, 19, 6, and 24 AM fungi species were found in the saline soil of Argentina, northern Portugal, and the salt marsh of Cabo de Gata Natural Park in Europe (Becerra *et al.*, 2014; Estrada *et al.*, 2013b; Oliveira *et al.*, 2005). In addition, 18, 33, and 26 AM fungal species were identified from the rhizosphere of plants in saline soils of Gansu, Ningxia, and Inner Mongolia Province, China, respectively (Huang, 2007; Liu *et al.*, 2017; Zhang, 2007). Importantly, the composition of AM fungal communities is strongly influenced by the high pH of salinized soils and is often specific in such soils, which may contain AM fungi with special functions, especially strong stress resistance (Carvalho *et al.*, 2003; Adenan *et al.*, 2021; Estrada *et al.*, 2013a). Therefore, exploring the composition of AM fungi population in the different rhizosphere of different plants in different areas is conducive to the development of professional AM fungal agents (He *et al.*, 2020).

Puccinellia tenuiflora, a perennial herbaceous plant of the Poaceae, is an excellent forage that grows well in soils with high Na^+ and pH (Greenway and Munns, 1980; Chen *et al.*, 2018; Zhao *et al.*, 2016), widely distributed in the Songnen saline grassland (Yu *et al.*, 2011). Yang *et al.* (2019) showed that the growth of *P. tenuiflora* could promote the downward leaching of salt and reduce the evaporation of water, which is important for the restoration of alkaline soil and is the pioneer of the restoration of degraded land in Songnen saline grassland. As for the salt-alkali tolerance mechanism of *P. tenuiflora*, many scholars have carried out a series of studies (Gao *et al.*, 2005; Wang *et al.*, 2008; Kobayashi *et al.*, 2015). More importantly, the degradation of saline-alkali soil has been controlled to some extent through the application of cultivated *P. tenuiflora* in Heilongjiang Province and other areas, which also confirms the higher tolerance and ecological value of *P. tenuiflora* (Sun *et al.*, 1997; Yan and Sun, 2000).

In an earlier study, we found that AM fungi positively affect the tolerance of *P. tenuiflora* under NaCl and $NaHCO_3$ stresses with a concentration of up to 400 mmol/L. That is, AM fungi inoculation can improve the resistance to stress to a certain extent by increasing the activity of antioxidant enzymes, the content of metabolites, and plant hormones in *P. tenuiflora* (Zhang and Yang, 2018; Yang *et al.*, 2020b). Therefore, it seems reasonable to use AM fungi to further improve the saline-alkali tolerance of *P. tenuiflora* and then use this symbiont in saline-alkali soil remediation. More investigations on AM fungi symbiotic with *P. tenuiflora* are worth carrying out. However, in our previous study, only three dominant AM fungi were identified in the rhizosphere soil of *P. tenuiflora* in songnen saline-alkali grassland by the molecular biological method, including *Rhizoglomus intraradices*, *Claroideoglomus etunicatum*, and *Funneliformis mosseae* (Yang *et al.*, 2020a). The extraction of AM fungal spore DNA is often affected by the composition of microflora and growth cycle, and the DNA is prone to contamination in the amplification process, making it difficult to obtain correct detection results (Clapp *et al.*, 2002). Therefore, the in-depth study of AM fungal diversity is still inseparable from the traditional morphological method for spore identification. Based on these, considering *P. tenuiflora* in Songnen saline-alkali grassland as the research object, in this study, we investigated the colonization of AM fungi in the rhizosphere of *P. tenuiflora*, isolated AM fungal spores in the rhizosphere soil, and identified the species and diversity of AM fungi symbiotic with *P. tenuiflora* through morphological characteristics. It is expected to provide a theoretical basis for studying AM fungal resources in saline-alkaline habitats and promoting the application of AM fungi in improving the saline-alkali tolerance of *P. tenuiflora*, especially for the strain screening in the production of mycorrhizal seedlings.

Materials and Methods

Sampling site and samples collection

Zhaodong City (125°–125°42' E, 46°16'–46°17' N) in Heilongjiang Province is located in the middle of the Songnen Plain and has a temperate continental monsoon climate. Precipitation is concentrated during specific times of the year, with annual precipitation of 350–550 mm, which primarily occurs from July to September.

Referring to the method described by Meng (1996), nine sites were randomly selected from four different directions, where large clumps of single species of *P. tenuiflora* were distributed. After removing the impurities of dead leaves and 5 cm thick topsoil, the soil was dug to a depth of 10–20 cm to collect 1.5 kg of the intact root system and rhizosphere soil of *P. tenuiflora* per site. Then, 1 kg of the sample was selected by quartering for air-drying and preservation. The roots were separated from each sample, cut into approximately 1 cm-long segments, and soaked in FAA solution (5 mL formalin, 5 mL glacial acetic acid, and 90 mL of 70% ethyl alcohol) after being repeatedly washed with distilled water. Finally, the remaining soil and roots samples were stored at 4°C.

Determination of soil pH

The soil pH was measured using a pH meter (METTLER TOLEDO FE20). From each sample, 20 g of air-dried soil was taken and fully dissolved in 20 mL distilled water, and the adjusted pH meter was immersed into the mixed suspension to read the pH value; this was repeated three times.

Assessment of natural colonization of arbuscular mycorrhizal fungi

Trypan-blue staining method was used to stain the structure of AM fungi in root segments. First, the root segments soaked in the FAA solution were taken out, washed with distilled water, and incubated in a 10% KOH solution (90°C, 60 min) to make them soft and transparent. After that, the root segments were neutralized for 5–10 min with 2% hydrochloric acid and then stained at 90°C for 30 min in 0.05% trypan-blue reagent. The decolorization was carried out with glycerin lactate solution. The morphology of the arbuscle, vesicles, and hyphae was examined under the microscope (OLYMPUS-DSX500), and 10 root segments were repeated for each sample. The colonization status of *P. tenuiflora* was evaluated and graded by the root segment observation method (Trouvelot *et al.*, 1986); that is, the

colonization intensities of the root segments were categorized into five grades: (1) 0%–1%: Grade 1, (2) 1%–10%: Grade 2, (3) 11%–50%: Grade 3, (4) 51%–90%: Grade 4, and (5) 91%–100%: Grade 5. Similarly, the arbuscular abundance was categorized into three grades: (1) Grade 1: the number of arbuscles was <5%, (2) Grade 2: the number of arbuscles was 5%–50%, and (3) Grade 3: number of arbuscles was >50%. The evaluation method of vesicles was the same. MYCOCALC software was used to enter grade parameters to obtain colonization rate (%), colonization intensity (%), arbuscular abundance (%), and vesicle abundance (%). The calculation formulae were as follows:

Colonization rate (%) = the number of colonized root segments/the total number of root segments × 100.

Colonization intensity (%) = $(0.95 \times N_5 + 0.70 \times N_4 + 0.30 \times N_3 + 0.05 \times N_2 + 0.01 \times N_1)$/total number of root segments × 100. Note: 0.95, 0.70, 0.30, 0.05, and 0.01 represent the weight of each grade, respectively, the same below. N_5 = the number of the root segments colonized at Grade 5, N_4, N_3, N_2, and N_1 had the same meaning.

Arbuscular abundance (%) = $(mA_3 + 0.5 \times mA_2 + 0.1 \times mA_1)$/100, where m = Colonization intensity (%) × the total number of root segments/the number of colonized root segments. Note: A_3 = the number of root segments with arbuscular abundance of Grade 3.

Separation and morphological identification of arbuscular mycorrhizal fungi spores

The spores were isolated by wet screening and sucrose density-gradient centrifugation method. Fifty grams of soil from each sampling point was passed through three standard soil sieves (the aperture from top to bottom is 76, 50, and 38.5 μm); the soil on the sieve was washed in running water until the filtrate was clear. The residue in the lower two sieves was transferred into a 50 mL centrifuge tube containing 60% sucrose solution, allowed to stand for 1.5 h, centrifuged at 4500 r/min for 20 min. The obtained supernatant was quickly poured onto the sieve (aperture 38.5 μm) and washed with water for 2 min to obtain AM fungal spores.

The spores were counted under the anatomical microscope. Spore density (SD) was calculated as the number of spores per 50 g of air-dried soil from direct counts. For the morphological identification of spore species, spores were picked under the anatomical microscope, and then polyvinyl alcohol-lactic acid-glycerine (PVLG), as well as Melzer's reagent was added to prepare a permanent slide. The diameter, color, surface decoration, and thickness of single spores were observed under an optical microscope (OLYMPUS-DSX500). Fungal species were identified based on original and recent species descriptions, as well as the pictures and species classification provided by INVAM (http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html).

Diversity indexes

Diversity indexes were calculated, including indexes of separation frequency, relative abundance, and importance value. The equations used to calculate these indexes were as follows (Yang *et al.*, 2011):

Separation frequency (F) = Occurrence frequency of certain species/total sample number × 100%

Relative abundance (RA) = Spore number of certain species/total quantity of AM fungal spores × 100%

Importance value (IV) = (F + RA)/2 × 100%

Data analysis

The diversity function of the "Vegan" package of R and RStudio (version 4.1.1) were used to calculate the diversity index of the four genera with the largest abundance. The "TreeMap" package and the "d3Tree" package were used to build the tree diagram. The genescloud platform (https://www.genescloud.cn) was used to make the chord diagram. The "Psych" package of R and RStudio was used to construct the correlation matrix between pH value and AM fungi distribution, as well as to assess the correlation between the colonization indexes. The decorana function in RStudio was used to examine the axis lengths of the community data, followed by RDA analysis to explore the relationship between pH and AM fungal communities. SPSS (Statistical Product and Service Solutions) 25.0 was used for one-way ANOVA and correlation analysis. Spearman correlation coefficient was used to describe the correlation, and when $p < 0.05$, the difference was statistically significant. Excel (2019) was used to process the experimental data, and all the data were expressed as mean ± standard deviation ($n = 3$).

Results

Natural colonization of arbuscular mycorrhizal fungi

A typical *Arum*-type (A-type) mycorrhizal structure was observed in the roots (Fig. 1). The structural characteristics of *P. tenuiflora* rhizosphere were as follows: the hyphae colonized on the root surface (Fig. 1a), grew in cortical cell spaces of the host plant, generated many intercellular hyphae (Fig. 1b), which could branch laterally into the cells (Fig. 1c). A portion of hyphae grew into dichotomous branching (Fig. 1e) and formed the arbuscules (Fig. 1d), a typical AM structures with a cauliflower shape. The apices of the endophytic hyphae expanded to form vesicles and were consistently presented as a circle (Fig. 1g), oval (Figs. 1h and 1i), or irregular shapes (Figs. 1c and 1f).

The investigation showed that the soil of the nine sampling sites in Songnen saline-alkali grassland belonged to severe alkaline soil (pH > 8.5, Table 1), and the ANOVA test indicated significant differences among different sites. The roots of *P. tenuiflora* could be colonized in different pH soil habitats, but the value of colonization indexes was not normally distributed. As shown in Table 1, when the soil pH was at the lowest value (pH = 9.35), the colonization rate was 80%, and the spore density was 25.14/g, reaching the maximum value. When the soil pH was the highest (pH = 9.81), the colonization rate was the lowest (40%). In addition, the maximum colonization rate (93.3%) was observed when the soil pH reached 9.72, the maximal vesicle abundance (13.31%), and the maximal colonization intensity (30.87%) were observed at a soil pH of 9.50. The values of arbuscular abundance were relatively low (<1%) in all examined samples, and even no arbuscule was detected in most samples.

The morphological identification of AM fungi

Through morphological identification, 40 AM fungi species belonging to 14 genera were isolated from the rhizosphere

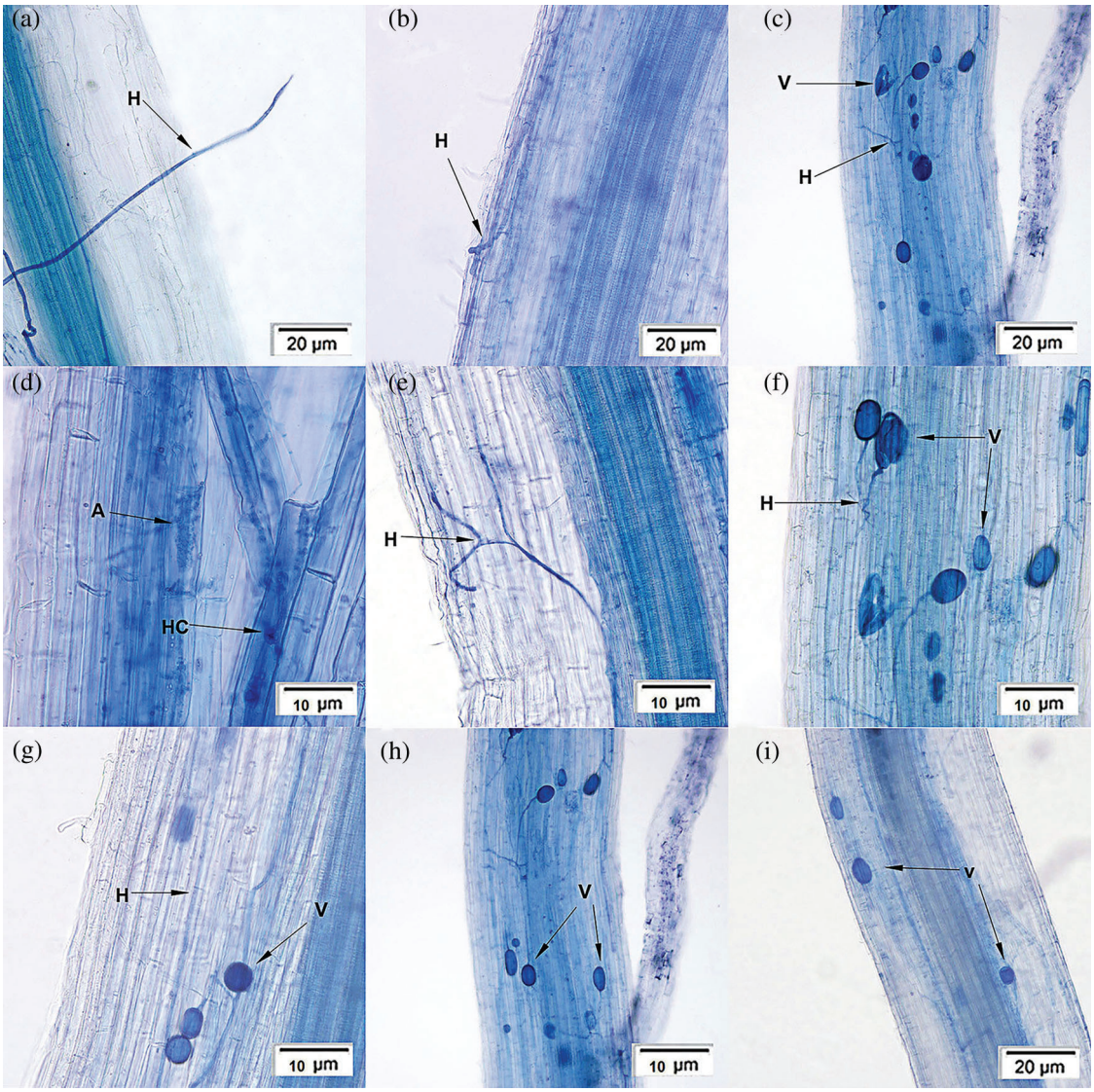

FIGURE 1. Symbiotic structure characteristics of *Puccinellia tenuiflora* rhizosphere. Notes: Hypha (H); Vesicle (V); Hypha coil (HC); Arbuscule (A).

TABLE 1

The colonization of arbuscular mycorrhizal (AM) fungi in the roots of *Puccinellia tenuiflora* in different sampling sites

Sample number	pH	Colonization rate/%	Colonization intensity/%	Arbuscular abundance/%	Vesicular abundance/%	Spore density/g	AM type
1	9.72 ± 0.04b	93.33 ± 0.64a	30.00 ± 1.74a	0.00 ± 0.00c	11.13 ± 1.55a	11.30 ± 3.21b	A
2	9.75 ± 0.02ab	60.00 ± 0.51d	22.60 ± 1.28b	0.00 ± 0.00c	11.65 ± 1.50a	14.48 ± 2.83b	A
3	9.59 ± 0.03c	60.00 ± 0.62d	4.30 ± 1.39e	0.00 ± 0.00c	0.05 ± 0.03c	11.92 ± 2.06b	A
4	9.80 ± 0.04a	60.00 ± 0.28d	19.80 ± 2.0bc	0.70 ± 0.05a	9.76 ± 1.40a	14.10 ± 1.92b	A
5	9.48 ± 0.04d	60.00 ± 0.14d	17.70 ± 1.18cd	0.00 ± 0.00c	12.05 ± 3.00a	12.24 ± 2.97b	A
6	9.69 ± 0.02b	64.29 ± 0.63c	21.36 ± 2.3b	0.00 ± 0.00c	1.56 ± 1.41bc	15.06 ± 1.88b	A
7	9.81 ± 0.02a	40.00 ± 0.53e	19.20 ± 0.46bc	0.00 ± 0.00c	4.75 ± 1.07b	12.54 ± 3.40b	A
8	9.50 ± 0.04d	66.67 ± 0.64c	30.87 ± 2.66a	0.00 ± 0.00c	13.31 ± 3.83a	14.14 ± 1.71b	A
9	9.35 ± 0.04e	80.00 ± 0.27b	15.60 ± 1.15d	0.60 ± 0.1b	1.41 ± 0.81bc	25.14 ± 2.52a	A

Note: Different letters within the same column indicate a significant difference among the sites at the 0.05 level.

soil of *P. tenuiflora* (Fig. 2), including 12 species of *Glomus*, 11 species of *Acaulospora*, five species of *Rhizophagus*, two species of *Ambispora*, as well as one species each of *Septoglomos*, *Funneliformis*, *Entrophospora*, *Diversispora*, *Sclerocystis*, *Scutellospora*, *Pacispora*, *Claroideoglomus*, *Racocetra*, and *Halonatospora*.

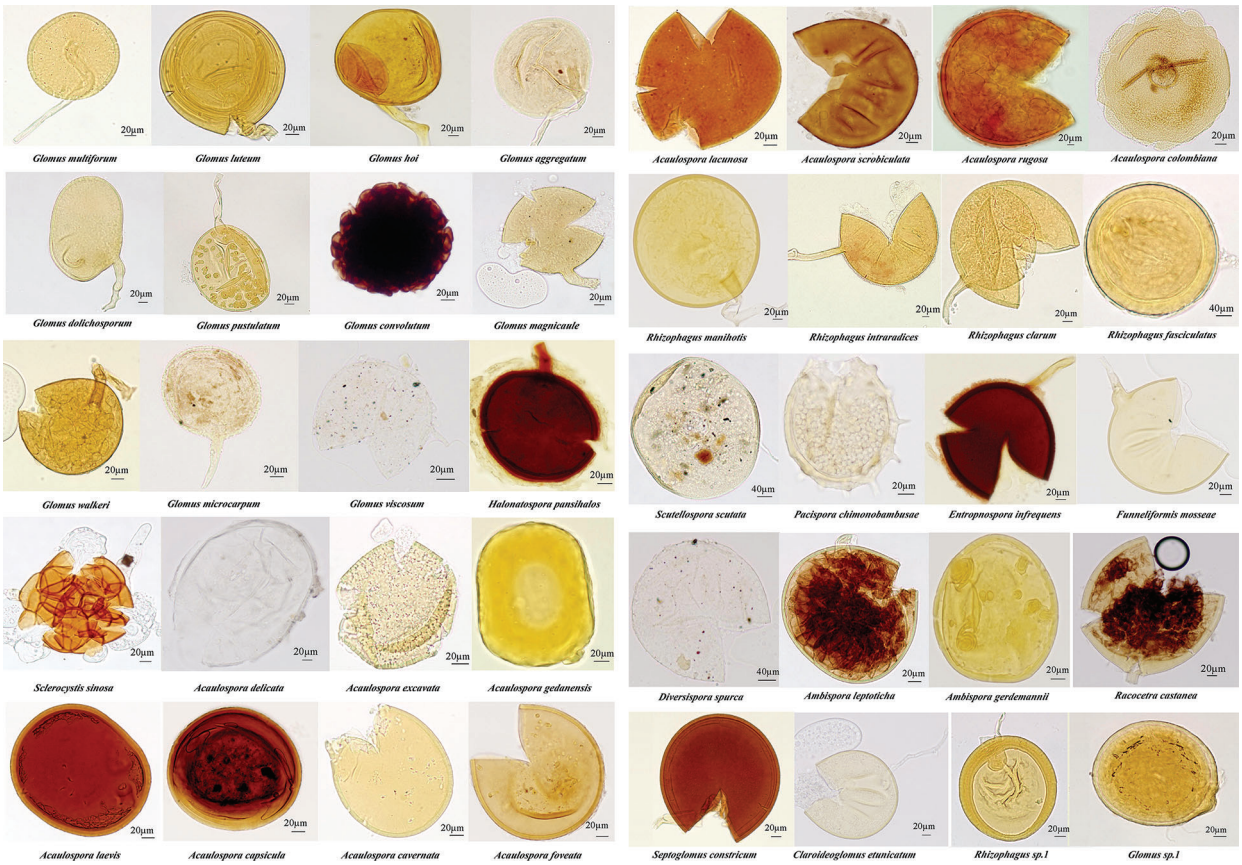

FIGURE 2. Spore morphology of arbuscular mycorrhizal fungi in the rhizosphere of *Puccinellia tenuiflora*.

The diversity indexes of arbuscular mycorrhizal fungal species

As shown in Table 2 and Fig. 3a, among the nine soil samples, the largest diversity were identified in Nos. 2, 1, and 9, with 20, 19, and 19 species, accounting for 50.00%, 47.50%, and 47.50% of the total, respectively. In addition, the complex relationship between the distribution and quantity of AM fungi and sampling sites can be seen in Fig. 3a, indicating a complex association network in the community, with a strong adoptive ability of the community to the changeable saline-alkali environment. As shown in Table 2 and Fig. 3b, *Glomus* was the most widely distributed genus with the highest richness, accounting for 30% of all species, followed by *Acaulospora*, *Rhizophagus*, and *Ambispora*. What's more, *Funneliformis mosseae* and *Rhizophagus intraradices* were isolated from all soil samples. *Acaulospora laevis*, *Acaulospora scrobiculata*, and *Claroideoglomus etunicatum* were common species in the rhizosphere of *P. tenuiflora* and were observed in all six soil samples. *Acaulospora colombiana*, *Acaulospora lacunosa*, and *Sclerocystis sinuosa* were occasional species and were only isolated from soil samples 3, 5, and 1, respectively.

Correlation analysis

Spearman correlation coefficient (Fig. 4) showed that vesicle abundance and arbuscular abundance were significantly correlated with colonization intensity at the level of 0.05.

The RDA analysis (Axis length = 1.9679) results showed a significant effect of the soil pH on the variation of AM fungal community between different sampling sites ($r^2 = 0.903$, $p = 0.001$).

In addition, Correlation matrix analysis showed that pH was correlated with the distribution of multiple species. For example, pH was negatively correlated with *Glomus pustulatum* (R = −0.693, $p = 0.039$), *Diversispora spurca* (R = −0.6928, $p = 0.039$), and *Glomus aggregatum* (R = −0.725, $p = 0.0272$). On the contrary, pH was positively correlated with *Rhizophagus clarum* (R = 0.693, $p = 0.0385$) and *Acaulospora foveata* (R = 0.822, $p = 0.007$).

Discussion

Arbuscular mycorrhizal fungi naturally colonized the root system of Puccinellia tenuiflora

As is well-known, the mycorrhizal colonization rate describes the colonization degree of plant roots by AM fungi and also reflects the arbuscular mycorrhiza formation and the affinity of AM fungi to plants. In the natural state, the high colonization rate may be due to the highly harmonious symbiotic relationship between AM fungi and host plants in the long–term coevolution process. The structures of arbuscle, hyphae, and vesicles observed in this study were similar to those of Moreira-Souza *et al.* (2003), and these structural characteristics are the main indicators for assessing mycorrhiza formation. Among them, vesicles usually exist in roots for a longer period, while the formation and decomposition of arbuscle are relatively fast (Wu *et al.*, 2009), which may be one of the reasons why the abundance of vesicles detected in all samples was higher than that of arbuscle. In addition, *P. tenuiflora* often grows in alkaline spots, where there is temporary shallow water accumulation on the surface in the rainy season (Zhao *et al.*, 2000). AM fungi are sensitive to excess water and hypoxia,

TABLE 2

The distribution and diversity indicators of arbuscular mycorrhizal (AM) fungi in the roots of *Puccinellia tenuiflora* in different sampling sites

Genera	Species	Sample number									Separation frequency (F, %)	Relative abundance (Ra, %)	Importance value (Iv, %)
		1	2	3	4	5	6	7	8	9			
Glomus	*multiforum*		+	+		+		+		+	55.56	1.87	28.71
	luteum	+					+	+	+		44.44	1.66	23.05
	hoi		+		+		+				33.33	1.69	17.51
	aggregatum					+				+	22.22	1.31	11.76
	dolichosporum	+			+			+	+		44.44	2.26	23.35
	pustulatum			+		+	+			+	44.44	1.21	22.83
	convolutum	+	+		+	+	+				55.56	1.59	28.57
	magnicaule		+	+					+	+	44.44	1.37	22.90
	walkeri	+							+		22.22	1.41	11.82
	microcarpum				+	+					22.22	1.34	11.78
	viscosum	+				+					22.22	1.30	11.76
Acaulospora	*delicata*		+	+				+			33.33	1.99	17.66
	excavata		+	+		+		+		+	55.56	1.39	28.47
	colombiana			+							11.11	2.23	6.67
	gedanensis		+		+						22.22	2.24	12.23
	laevis	+	+		+	+		+		+	66.67	2.34	34.51
	capsicula	+	+		+					+	44.44	1.41	22.92
	cavernata	+		+		+				+	44.44	1.52	22.98
	foveata		+		+			+			33.33	1.57	17.45
	lacunosa					+					11.11	1.61	6.36
	scrobiculata	+	+	+		+	+	+			66.67	1.90	34.28
	rugosa			+		+					22.22	1.92	12.07
Rhizophagus	*fasciculatus*	+				+	+			+	44.44	1.99	23.22
	manihotis	+		+			+				33.33	1.62	17.48
	intraradices	+	+	+	+	+	+	+	+	+	100.00	12.61	56.30
	clarum	+			+		+	+			44.44	2.36	23.40
Ambispora	*leptoticha*		+		+		+			+	44.44	2.08	23.26
	gerdemannii		+							+	22.22	2.29	12.25
Septoglomus	*constricum*	+	+					+		+	44.44	1.91	23.17
Funneliformis	*mosseae*	+	+	+	+	+	+	+	+	+	100.00	15.95	57.97
Entrophospora	*infrequens*	+		+			+	+			44.44	1.72	23.08
Diversispora	*spurca*	+				+			+	+	44.44	2.34	23.39
Sclerocystis	*sinuosa*	+									11.11	1.99	6.55
Scutellospora	*scutata*	+	+								22.22	2.11	12.17
Pacispora	*chimonobambusae*			+	+			+	+	+	55.56	2.02	28.79
Claroideoglomus	*etunicatum*		+	+	+		+		+	+	66.67	5.59	36.13
Racocetra	*castanea*			+		+					22.22	1.61	11.92
Halonatospora	*pansihalos*		+					+	+		33.33	1.47	17.40
Glomus	*Sp.1*		+			+				+	33.33	1.66	17.50
Rhizophagus	*Sp.1*			+						+	22.22	1.57	11.90

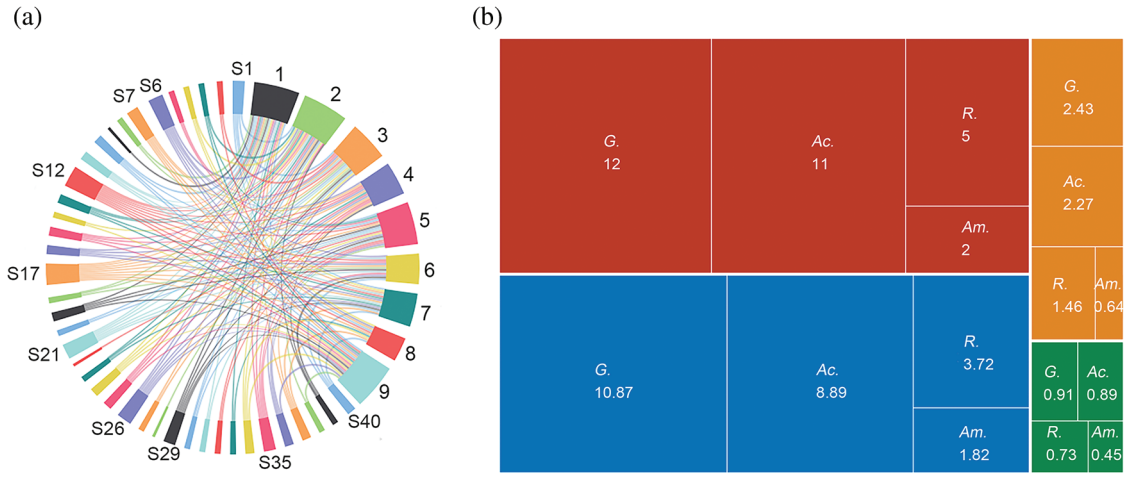

FIGURE 3. Arbuscular mycorrhizal (AM) fungal species distribution (a) and Alpha diversity of the four most abundant genera (b). Note: (a), S1–S40: 40 AM fungal species (The numbering of some species is omitted); 1–9: 9 sample sites. The proportion of each node segment shows the overall proportion of the number of species or sample sites. (b), Red area: Total species; Blue area: Inv; Orange area: Shannon index; Green area: Simpson index; G.: *Glomus*; *Ac.*: *Acaulospora*; *R.*: *Rhizophagus*; *Am.*: *Ambispora*.

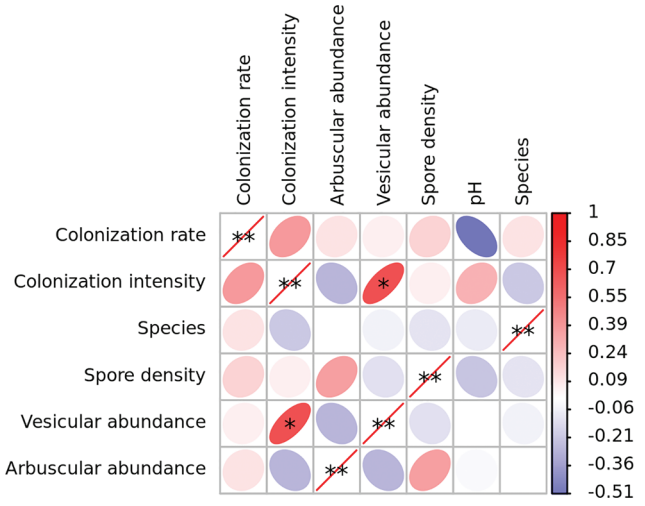

FIGURE 4. Correlations between pH, arbuscular mycorrhizal (AM) fungal colonization, and diversity in rhizosphere soil of *Puccinellia tenuiflora*. * indicated the significance at $p < 0.05$ and $R > 0.6$.

which is also an important factor that inhibits the formation of arbuscle.

Interestingly, we found a significant correlation between vesicle abundance and colonization intensity, possibly because vesicles, as nutrient storage organs (Yang *et al.*, 2015), play a unique role in the mycorrhizal colonization of plant roots (Smith and Read, 2008).

Arbuscular mycorrhizal fungi resources are abundant in the rhizosphere of P. tenuiflora of Songnen saline-alkaline grassland

Recent studies have shown that the species richness of Glomeraceae is higher in saline soils (Estrada *et al.*, 2013; Krishnamoorthy *et al.*, 2020), and *Glomus* is the dominant genus (Bonfim *et al.*, 2016; Wang *et al.*, 2004). Our study also confirmed the conclusion. The spore-forming patterns of *Glomus* species may be more adaptive to environmental conditions in long–term saline stress, or soil nutrient and climate change enhance the dominance of *Glomus* (Bonfim *et al.*, 2016).

According to statistics, *Funneliformis mosseae* and *Rhizophagus intraradices*, were the most widely distributed species in the rhizosphere of *P. tenuiflora*, and these two species were also found in saline habitats of many other countries (Estrada *et al.*, 2013b; Evelin *et al.*, 2012; Oliveira *et al.*, 2005), which suggests that these two species possibly have a higher level of adaptability. Besides, our previous studies have shown that both *Funneliformis mosseae* and *Rhizophagus intraradices* formed a symbiotic association with *P. tenuiflora* and improved the salt-alkali tolerance of *P. tenuiflora* (Yang *et al.*, 2017; Zhao *et al.*, 2020). Moreover, previous studies have verified the effects of *Funneliformis mosseae* and *Rhizophagus intraradices* on the salt tolerance of plants (Wang *et al.*, 2020; Qiu *et al.*, 2020). By increasing the nutrient content in roots and leaves (e.g., N, P, K, Ca, and Mg), maintaining a good ion balance (e.g., K^+/Na^+), increasing the activity of antioxidant enzymes, and the accumulation of antioxidant compounds in plants, mycorrhiza could improve the tolerance of plants to salt stress. Therefore, it could be preliminary concluded that *Funneliformis mosseae* and *Rhizophagus intraradices* might show a certain degree of competitiveness and efficiency in a saline-alkali environment.

Notably, among the 40 AM fungi species isolated in our study, the two species, which distributed widely, belonging to *Glomus* and *Rhizophagus*, respectively, could not be identified and needed to be propagated for further identification. In addition, the abundant AM fungi resources distributed in Songnen saline-alkali grassland need to be explored further in the future.

Effects of high pH of saline-alkali soil on arbuscular mycorrhizal fungi

Previous studies on species composition of AM fungi in saline soils showed that soil pH directly affects the species distribution and sporulation of AM fungi, as well as the formation and effectiveness of mycorrhiza (Yang *et al.*, 2020c; Wang *et al.*, 2010). However, it should be noted that in the current study, we did not find any significant association of pH with AM fungal colonization, species richness, and spore density.

However, it had a significant impact on the distribution of partially AM fungal species; this might be related to the adaptability of these AM fungal species formed in the long-term evolution, making them more vulnerable to the influence of alkalinity, and the impact of other factors needs to be further studied.

Conclusions

This study showed abundant AM fungal resources in the rhizosphere of *P. tenuiflora* growing in Songnen saline-alkaline grassland. AM fungi colonize the roots of *P. tenuiflora* to form *Arum*–type arbuscular mycorrhiza, and the colonization intensity is significantly correlated with the abundance of vesicle structure. Genus *Glomus* had the widest distribution and the dominant position. *Funneliformis mosseae* and *Rhizophagus intraradices* might be the efficient AM fungal species in saline-alkali habitats. High soil pH value only affected the distribution of some AM fungi and had no significant correlation with colonization, species richness, and spore density. These findings provide a theoretical basis for further understanding of the highly-efficient AM fungal resources in saline-alkali soils and promote the application of mycorrhizal symbionts in the ecological restoration of saline-alkali lands. However, the molecular diversity of AM fungi in Songnen saline-alkali grassland and the role of soil physical and chemical properties on diversity needs further exploration and will be the focus of our future research.

Availability of Data and Materials: All data generated or analyzed during this study are included in this published article.

Author Contribution: The authors confirm contribution to the paper as follows: study conception and design: Chunxue Yang; data collection: Fei Chen; analysis and interpretation of results: Yajie Liu, Fei Chen; problem-solving of the research: Wenna Zhao, Yudan Wang; draft manuscript preparation: Yunhui Zhou. All authors reviewed the results and approved the final version of the manuscript.

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