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EFFECTS OF *CENOCOCCUM GEOPHILUM* ON THE GROWTH OF SEEDLINGS OF *OSTRYOPSIS DAVIDIANA* DENCE. AND *PINUS TABULEAFORMIS* CARR.

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Abstract

The affinity and promoting ability of the *Cenococcum geophilum* strains ($CgSO_1$, $CgSB_2$, CgO_5, $SPOP_2$ and $Cg_5^{\#}$) to *Ostryopsis davidiana* and *Pinus tabulaeformis* were studied. The results showed that the tested strains formed ectomycorrhizae（ECM）with *O. davidiana* except the $Cg_5^{\#}$ strain and the infection rates were 40-50%. $CgSO_1$ and $CgSPOP_2$ formed ECM with *P. tabulaeformis* and the infection rates were 10.3 and 12.4%, respectively. *C. geophilum* can promote the growth of the two host plant seedlings, especially for root growth. Results proved that the affinity of different strains to host plants and the affinity of the same strain to different host plants are different.

Introduction

Daqing Mountain located in Inner Mongolia is undergoing forest restoration about 400 kilometers west of Beijing, but it is a buffer zone for sandstorms. Therefore, the current vegetation degradation is still serious. *Ostryopsis davidiana* and *Pinus tabulaeformis* are widely distributed in the harsh site conditions of Daqing Mountain. *O. davidiana* belonging to Betulaceae is a special excellent soil protection shrub in China. *P. tabulaeformis* is a conifer tree species endemic to China and widely distributed in temperate forests of north China (Wang *et al*. 2017).

Bai *et al.* (2009) reported that it is difficult for the transplanted seedlings to survive in the absence of mycorrhiza fungi. Compared with fixed-planting seedlings, non-mycorrhizal seedlings lacked drought tolerance and reduced absorption to water and soil nutrient. Moreover, colonization of *O. davidiana* by *Cenococcum geophilum* was greater than 50% when the *P. tabulaeformis* in the two trees mixed forest. This suggests the possibility that *C. geophilum* has higher affinity with *O. davidiana* than with *P. tabulaeformis* (Yao *et al.* 2017).

Cenococcum geophilum is common ectomycorrhizal fungus with widespread distribution in boreal or temperate habitats and connected with variety of spermatophyta hosts (De *et al.* 2018, Maíra *et al*. 2018). Furthermore, *C. geophilum* is the only ectomycorrhizal species appertain to the clade Dothideomycetes (Obase *et al.* 2016). Recent studies have shown that mycorrhizal fungi is one of pathogenic fungi, and use effectors with a small secreted protein (SSPs) as the molecular keys to promote symbiosis between plants and fungi (Kamel *et al.* 2017). The species of bacterial classes on the surface of ectomycorrhizae root system were significantly more than that of non-ECM root system (Sakoda *et al.* 2018). *C. geophilum* is currently recognized as an ectomycorrhizal fungus with strong drought tolerant character (Fernandez and Koide 2013), and it is also one of the most indigenous ectomycorrhizal fungi in Inner Mongolia (Yao *et al.* 2017).

In the present study, five *C. geophilum* strains were used to inoculate *O. davidiana* and *P. tabulaeformis* seedlings. This study would provide the theoretical basis for vegetation restoration afforestation of Daqing Mountain and the foundation of mycorrhizal biological application technology in Inner Mongolia.

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Materials and Methods

Daqing Mountain is located in the middle of the Yinshan Mountains (Long 109º46´E to113º04´E, Lat 40º34´N to 41º18´N) in central Inner Mongolia. *O. davidiana* Seeds were collected from healthy trees on Daqing Mountain (1,600 m) whereas *P. tabulaeformis* seeds were purchased from a commercial seed company in Inner Mongolia.

$CgSO_1$, $CgSB_2$ and $CgSPOP_2$ were isolated from *C. geophilum* sclerotia in the rhizosphere soil of *O. davidiana*, *Betula platyphylla and Populus davidiana*. CgO_5 was isolated from the ectomycorrhizal root tissue of *O. davidiana*. $Cg_5^{\#}$ was introduced from France.

C. geophilum strains inoculants and *O. davidiana* and *P. tabulaeformis* seedlings for testing were prepared using the method described by Bai *et al.* (2009).

Six levels (five strains and one control) were tested ten times. Approximately 30 ml of the fungi agent was then inoculated into the root system. In the Control (no live fungi inoculation) 6 ml of inoculum was taken per strain, mixed, sterilized at 121°C for 60 min, and added to the seedling side. All containers were randomly placed in a growth chamber, cultured in the dark for one day, and placed in a light culture chamber (23 to 25°C). Hoagland nutrient solution were applied every 30 days to ensure the necessary nutrition of the seedlings.

Six months after transplanting, the seedlings of every 5 treatments were randomly selected to observe the mycorrhizal infection rate. Mycorrhizal infection rate (%) = (mycorrhizal number/total root number) × 100. The stem diameter and biomass were measured. The stem diameter and biomass of the root ring were measured. At the same time, according to the list given by Ageer (1999, 2006) the morphological and anatomical characteristics of mycorrhiza are described. Two-way analysis of variance (ANOVA) was used to determine whether *C. geophilum* significantly affected the influencing variables (ECMF infection rate, height, stem diameter, and dry weight) of seedlings.

Results and Discussion

After 6 months of inoculation, the unique mycorrhizae morphology for each strain on *O. davidiana* seedlings was studied. The infection rates were 45 - 48.1%, all reached the fourth-level infection rate. The mycorrhizae morphology were studied for only $CgSO_1$ and $SPOP_2$ strains on *P. tabulaeformis* seedlings, while the infection rates were only 10.3 and 12.4% respectively. The results highlighted that the *C. geophilum* colonization rate of *O. davidiana* and *P. tabulaeformis* seedlings were dependent on the different ectomycorrhizal fungi strains.

From Figs 1 and 2 it was apparent that $CgSPOP_2$ and $CgSO_1$ strains formed typical ectomycorrhizal fungi. Moreover, the mycorrhizae morphology on *P. tabulaeformis* and *O. davidiana* was similar. Morphology of *O. davidiana* (Fig. 1A) and *P. tabulaeformis* (Fig. 2A) is hydrophilic, monopodial-pinnate or unramified, blank, 4 - 6 mm long, 0.4 - 0.6 mm diam., unramified ends inflated, with abundant dark rigid emanating hyphae, no rhizomorph, with rigid mycelium radially projecting from the surface, not smooth, shiny.

Mantle in plain view of *O. davidiana* (Fig. 1 B and C) and *P. tabulaeformis* (Fig. 2B and C) is plectenchymatous mantles, hyphae star-like arranged and tightly glued together (Type G), membranaceously brownish, and cell wall thick (4.0 - 6.0 μm), with simple septa.

Effects of adding five *C. geophilum* strains on the response variables of *O. davidiana* seedlings were significant ($p < 0.05$). The height of mycorrhizal seedlings increased by 49.6 - 65.9%. The stem diameter increased by 14.9 - 21.9%. The number of lateral roots increased by 21.7 -34.8%. The dry weights of the seedlings of the aboveground portion increased by 51-66%. The dry weights of the seedlings of the underground portion increased by 108.1 - 135.5%. The

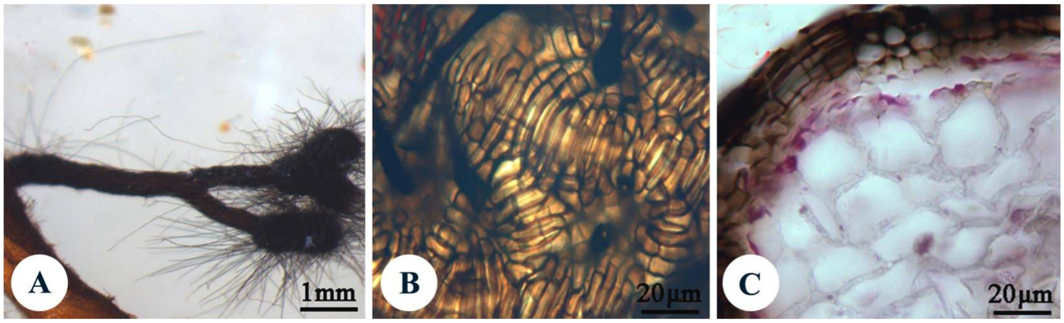

Fig. 1. Morpho-anatomical features of ectomycorrhizae on roots of *Ostryopsis davidiana.* A: External morphological features; B: Plan view of ectomycorrhizal mantle; C: Transverse section of ectomycorrhiza

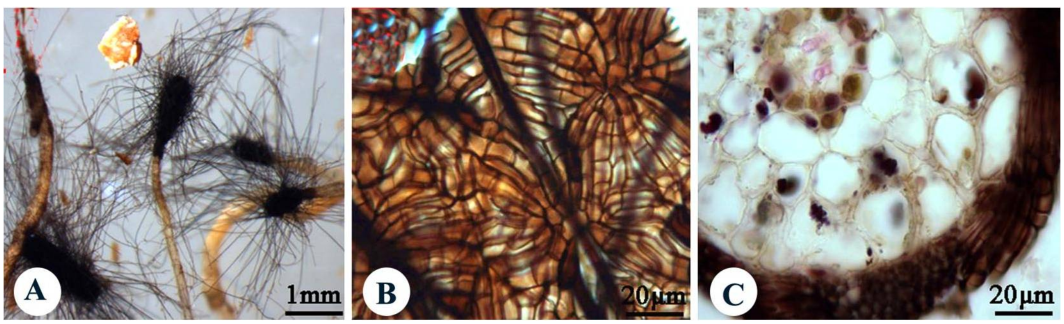

Fig. 2. Morpho-anatomical features of ectomycorrhizae on roots of *Pinus tabulaeformis*. A: External morphological features; B: Plan view of ectomycorrhizal mantle C: transverse section of ectomycorrhiza

increase in total dry weight is all around 80%. The ratio of root to shoot ratio increased significantly. The relative water content increased in the range of 4.9 to 8.5% (Table 1).

Table 1. Effects of *Cenococcum geophilum* on the Growth of seedlings of *Ostryopsis davidiana.*

Treatment	Height (cm)	Stem diameter (mm)	Lateral root numbers	Dry mass(g)	root-shoot ratio(mass/ mass)	Relative water content	ECMF infection rate (%)
$CgSO_1$	19.8^a	2.78^a	31^a	1.661^a	0.835^a	89^a	45
$CgSB_2$	19.3^a	2.72^a	28^a	1.515^a	0.789^a	87^a	46.8
$CgSPOP_2$	21.4^a	2.62^a	28^a	1.608^a	0.727^a	88^a	48.1
CgO_5	20.8^a	2.62^a	29^a	1.657^a	0.738^a	86^a	49.2
$Cg_5^{\#}$	16^b	2.34^a	24^b	0.976^b	0.587^b	84^b	0
CK	12.9^b	2.28^a	23^b	0.882^b	0.572^b	82^b	0

Different letters indicate significant differences ($p < 0.05$).

As with *P. tabulaeformis* seedlings, $CgSPOP_2$ and $CgSO_1$ had different effects. The height of the mycorrhizal seedlings increased by 8.6 and 7.6%. The stems diameter increased by 10.5 and 11.8%. The number of lateral roots increased by 12.5 and 18.8%. The total dry mass increased by 16.6 and 16.8%. The root-shoot ratio (mass/mass) increased by 20.7 and 22% (Table 2).

The present experiments showed that the mycorrhizal seedlings of *O. davidiana* and *P. taebulaeformis* grew better than non-mycorrhizal seedlings. However, compared with the exotic strains, the *O. davidiana* seedlings prefered to infect by native *C. geophilum* strains. Only the $CgSO_1$ and $CgSPOP_2$ strains infected the seedlings of *P. tabulaeformis* and the infection rates were low. This indicates that the affinity of *C. geophilum* and *P. tabulaeformis* was lower. On the other hand, it indicates that the strains from different environments had certain affinity to the same host. Because there are many factors which affect the mycorrhizal synthesis. The improvement of the colonization rate of *C. geophilum* to *P. tabulaeformis* remains to be further studied.

Table 2. Effects of *Cenococcum geophilum* on the Growth of seedlings of *Pinus tabulaeformis*.

Treatment	Height (cm)	Stem diameter (mm)	Lateral roots number	Dry mass(g)	Root-shoot ratio(mass/ mass)	Relative water content (%)	ECMF infection rate (%)
$CgSO_1$	8.6^a	1.88^a	18^a	0.631^a	0.582^a	97^a	10.3
$CgSB_2$	7.9^b	1.66^b	16^b	0.571^b	0.513^a	95^a	0
$CgSPOP_2$	8.5^a	1.90^a	19^a	0.632^a	0.588^a	98^a	12.4
CgO_5	8.1^b	1.68^b	16^b	0.545^b	0.482^a	96^a	0
$Cg_5^{\#}$	8.1^b	1.66^b	17^b	0.555^b	0.511^b	97^a	0
CK	7.9^b	1.70^b	16^b	0.541^b	0.482^b	96^a	0

Different letters indicate significant differences ($P < 0.05$).

There were significant differences in seedling growth and mycorrhizal colonization rates of different tree species inoculated with different *C. geophilum* strains. Both $CgSO_1$ and $CgSPOP_2$ strains formed mycorrhizal structure with *P. tabulaeformis* and *O. davidiana*, and the colonization rate of *O. davidiana* was significantly higher than that of *P. tabulaeformis*. This is in consistent with the results of the previous field survey, indicating that the *O. davidiana* species have a stronger dependence on *C. geophilum* (Yao *et al.* 2017).

It was observed that the mycorrhizal colonization rate is influenced by the two factors of the host plants and the ECM fungi strains. The affinity and dependence of different plant species on different mycorrhizal fungi are very different. Therefore, in the process of afforestation, the best combination of tree species and mycorrhizal fungi should be screened firstly (Vopravil *et al.* 2014). Therefore, the *C. geophilum* strains are suitable for forestation and afforestation, which will have a profound impact on the construction of plantation in China, provide a scientific basis for the theory and practice of vegetation restoration, and has a strong promotion and application value.

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