Development status of **Arbuscular mycorrhizal fungi** associated with invasive plant **Coreopsis grandiflora** Hogg.

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According to the distribution of **Coreopsis grandiflora** Hogg., samples were collected from two natural invasion sites; Jufeng and Beijiushui and cultivated site to research AM fungal arbuscule structure of **C. grandiflora** Hogg., colonization status in different sample plots and altitudes, and the correlation between colonization status and soil factors in the rhizosphere of **C. grandiflora**. The result showed that **C. grandiflora** could form heavy **Paris**-type arbuscule structure, Beijiushui mycorrhizal colonization percentage and numbers of entry points was highest and significant differences with Jufeng and campus. **C. grandiflora** Hogg. mycorrhizal colonization percentage was on the rise with rising altitudes. In addition to altitudes, soil factors as well as to influence AM fungal colonization status, there were very positive correlation \((r=0.792)\) and positive correlation \((r=0.725)\) between mycorrhizal colonization percentage and the content of organic carbon, available potassium in soil, respectively; there were very positive correlation \((r=0.854)\) and very negative correlation \((r=-0.966)\) between numbers of vesicles **P**-type arbuscule and the content of available potassium, pH in soil; there were negative correlation \((r=-0.782)\) between numbers of entry points and the content of available phosphorus. High infection rate and the formation of typical arbuscular form of **C. grandiflora** Hogg. at Laoshan should have an important role on the process from the invasion to becoming constructive species.

**Key words:** **Coreopsis grandiflora**, Arbuscular mycorrhiza fungi (AM fungi), mycorhizae structure, soil factors.

**INTRODUCTION**

Arbuscular mycorrhiza fungi (AM) are major component of soil microbial communities in terrestrial ecosystems, which can form mutually beneficial symbiosis with 90% of vascular plants (Liu and Chen, 2007). A mycorrhizal structure, such as infection conditions, mycelial growth and development, is the material basis of function of arbuscular mycorrhizal symbiosis. Gallaud firstly proposed two types of arbuscular mycorrhizal in 1905, later arbuscular mycorrhizal were divided into three types: **Arum**-type (**A**-type), **Paris**-type (**P**- type) and **Intermediate**-types (**I**- type) (Smith and Smith, 1997). Today arbuscular mycorrhizal studies of Compositae are mostly focused on the common cultivated crops, such as **Helianthus annus** and wild plants **Neopallasia pectinata**, **Artemisia argyi**, **A. eriopoda** and **Taraxacum mongolicum** (Muthukumar and Tamilselvi, 2010; Bao and Yan, 2004) study on arbuscular type of invasive plants in Asteraceae has been rarely reported. In addition, the ability of AM fungi to form symbiotic associations with host plants and mycorrhizal effects are relative to many environmental factors, including elevation and soil factors. The significant changes of altitudes, temperatures and precipitation have important implications on plant growth and AM fungi infection (Cai et al., 2005). Supply of soil nutrients plays an important role on mycorrhizal infection and the formation of the structure of AM fungi; currently the effects of the elevation and soil nutrients on the structure

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formation and growth of AM fungi have not reached the same result, and still lack a comprehensive and systematic study (Feng, 2003; He et al., 2010).

C. grandiflora Hogg., which has been identified as invasive species in China (Xu and Qiang, 2004) has spread in Jufeng of Laoshan and Beijiushui and become a serious threat to local ecosystems. Walling and Zabinski (2006) proved that the hyphae in rhizosphere of invasive plant Centaurea maculosa was higher than native plants compared with the indigenous plant, roots of Sapium sebiferum are rich in AMF (Nijjer et al., 2008); high infection rate of arbuscular has an important role on the process from the invasion to becoming constructive species. In this paper, AM fungal colonization in C. grandiflora Hogg. from Laoshan region was studied preliminarily, which will provide a theoretical basis for research and control of this invasion plant.

MATERIALS AND METHODS

Natural conditions of experimental site

Laoshan of Qingdao city brink of the Yellow Sea belongs to a temperate maritime climate, where the average annual temperature is 12.6°C, the average annual rainfall is 700 ~ 800 mm, and frost-free period is 180 ~ 200 days. The soil of experimental site is brown soil and slightly acidic. Vegetation is mainly Pinus thunbergii, Pinus densiflora, Robinia pseudoacacia, Alanthus altissima, Pistacia chinensis and Ulmus macrocarpa; Spiraea shrub species mainly includes Spiraea fritschiana, Prunus japonica, Zanthoxylum bungeanum, Spiraea trilobata, Rubus chororifolius and Lauraceae obtusiloba; Herbaceous species mainly includes Arrhenatherum elatius, Adenophora axilliflora, Lilium tsingtauense, Veratrum nigrum, Thalictrum minus var. hypoleucum and Polygonatum odoratum.

Sample collection

According to the distribution of C. grandiflora Hogg., samples were collected from two natural invasion sites Jufeng (Sunrise side of Laoshan Mountain, Altitude 12 ~ 426 m) and Beijiushui (nightside of Laoshan Mountain, Altitude 81 ~ 450 m) and cultivated site (Qingdao Agricultural University campus). Removed the topsoil, take the roots and rhizosphere soil of C. grandiflora Hogg., samples were placed in ziplock bag and registered, recorded collection time and altitude of sampling location by GPS. Each sample point was set 3 to 4 replicates.

Samples were taken back to the laboratory, the roots were put into the refrigerator for determination of mycorrhizal colonization; and soil samples were air-dried and screened after a 20 mesh sieve in the laboratory for determination of soil chemical properties.

Observation of mycorrhizal morphology

Mycorrhizal morphology was observed by acid fuchsin staining (Kormanik et al., 1980), plant roots were mixed every sampling point at one altitude, rinsed with distilled water and cut into 0.5 ~ 1.0 cm segments, which were put into test tubes with 5 ~ 10% KOH solution, then the tubes were put into water bath pot at 90°C for 15 min, then rinsed with distilled water and soaked in 2% HCL solution for 5 min; removed the solution and added 0.01% acid fuchsin lactic glycerol. staining solution at room temperature overnight; observed the samples with an optical microscope after separation by lactic acid.

We determined the morphology of mycorrhizae (arbuscular, vesicles and hyphae) according to Smith and Smith (1997) method; calculated the numbers of laps, vesicles and penetrated several points of mycelium of 100 root segments from every sampling sites. Mycorrhizal infection rate was calculated with the root segment frequency conventional method of Biermann and Linderman (1981), as the following formula:

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\text{Colonization} = \frac{\text{total number of root segments colonized}}{\text{total number of root segments studied}} \times 100
\]

Colonization of AM fungi from different sites

Colonization of AM fungi from different sites was showed in Figure 2. Mycorrhizal infection rate of C. grandiflora Hogg. From Beijiushui is highest, an average of 51.4%,
Figure 1. Mycorrhizal structure of *Coreopsis grandiflora*; 1. Entry points (EP); 2, 3, 4. External hyphae (EH); 5. Internal hypha (IH); 6, 7, 8. Paris arbuscule (A); 9. Spore (S); 10, 11, 12. Vesicle (V) Bars: 1, 2, 3, 4, 5, 9, 10 mean 100 µm; 6, 7, 8, 11, 12 mean 50 µm.

Figure 2. Development status of Arbuscular mycorrhizal fungi of *Coreopsis grandiflora* in different sample plots. LSD multiple comparisons in difference sample plots, significant differences in each column are indicated by different letters (*P* ≤ 0.05). MCP=Mycorrhizal colonization percentage, NHC=Numbers of hyphal coils, NV=Numbers of vesicles, NEP=Numbers of entry points.
which was significantly higher than Jufeng and campus; mycorrhizal infection rate from Jufeng (30.2%) was higher than from campus, but the difference was not significant. The numbers of vesicles in roots from three sites were significantly different, Jufeng 29.60/100 mm, Beijiushui 16.08/100 mm and the campus only 4.90/100 mm. The numbers of intrusion points of 3 sites were also significantly different, Beijiushui 9.31/100 mm, Jufeng 4.35/100 mm and campus 2.29/100 mm, but mycelium rings had no significant difference between each other, Beijiushui 7.58/100 mm, Jufeng 7.51/100 mm and campus 3.18/100 mm.

The impact of altitude on development status of AM fungal and correlation analysis

Mycorrhizal infection rate of C. grandiflora Hogg. increased with the altitude upward. Maximum of infection rate in Jufeng site is 95.6% at altitude from 339 to 426 m; minimum is 8.3% at an altitude of 12 m; maximum of infection rate in Beijiushui site is 74.7% at altitude from 337 to 348 m; minimum is 15.5% at an altitude of 144 m (Figure 3). Correlation analysis showed that the infection rate and altitude was positively correlated and numbers of vesicles, invasive point and hyphae turns was negatively correlated to altitude, but both did not reach a significant level. In addition, only numbers of hyphae laps and vesicles were significantly positively correlated ($r=0.842$), the other indicators had no significant correlation (Table 1).

The effects of soil factors on development status of AM fungal

The results showed that infection rate was significantly positively correlated to soil available potassium ($r=0.725$), and highly significantly positively correlated to soil organic matter content ($r=0.792$); the number of mycelium rings was significantly positively correlated to the content of available potassium ($r=0.854$), and was highly significantly negatively correlated to soil pH ($r=-0.966$); the number of intrusion points was significantly negatively correlated to soil available phosphorus content ($r=-0.782$) (Table 2).

DISCUSSION AND CONCLUSION

The results of this study showed that C. grandiflora Hogg. has a strong symbiotic relationship with mycorrhizal fungi, a large number of mycorrhizae can replace roots for...
absorption and competition for nutrients from the near native plants to result imbalance of plant transfer of nutrients (Berta et al., 1993; Vance et al., 2003; Carey et al., 2004). However, Pringle et al. (2009) proved that non-mycorrhizal invasive plants are also able to successfully invade new habitat (Ahulu et al., 2005). For example, Callaway et al. (2008) have found that the secretions of non-mycorrhizal onion mustard after invasion in North America disrupted the symbiotic relationship of native plants and AM fungi plant invasion, which reduced the growth and competitiveness of native plants, and ultimately produced a positive feedback on invasive plants. This study found that arbuscular of *C. grandiflora* Hogg. is P-type, that is, the AM fungi forms the typical mycelium root ring structure within the root. Cavagnaro et al. (2001) believed that the formation of P-type arbuscular takes longer time than the A-type, because mycelium root ring formation time take time. Morphology of A-type and P-type is relative to groups of plant succession, from the Pioneer group, early succession group to late succession group, the ratio of A-type arbuscular mycorrhizal reduced in the order and the P-type arbuscular mycorrhizal increased in the order 1. Imhof and Weber (1997) proved that P-type structure is a relatively advanced type at stress and low nutrient conditions because mycelium root ring may be a signal controlled by plant. Brundrett and Kendrick (1990a and b) speculated that the slow P-type AM colonization was beneficial for mycorrhizal host plants to maintain a low energy supply, which is conducive to growth and development of plant. *C. grandiflora* Hogg. is a perennial herb and has longer growth cycle, so the longer invasion period and mycelial growth period provide the basis for the formation of mycelium root ring. The formation of P-type arbuscular is favorable for the growth and competition of *C. grandiflora* Hogg. in the invasion habitat.

This study found that soil organic matter content and the infection rate has a significant positive correlation, indicating that soil organic matter content is conducive to the development of mycorrhizal fungi; and soil pH was negatively correlated to mycorrhizal infection rate and significantly influenced the formation of P-type arbuscular, which is consistent with the results of previous research (He et al., 2005). Nitrogen and phosphorus contents were negatively correlated with the invasion points, especially phosphorus content was highly significantly negatively correlated, proved that high phosphorus content can inhibit formation of intrusion points and growth of hyphae outside the root. Mycorrhizal growth and development requires a certain amount of potassium, an appropriate increase in potassium content is beneficial for the infection and the symbiotic formation of AM fungi (Li et al., 2002). Soil available K content was significantly positively related to mycorrhizal infection rate of *C. grandiflora* Hogg. and had the greatest impact on arbuscular. Correlation study is a preliminary exploration of the impact of a single soil factors on mycorrhizal colonization status, there may be some interaction to mycorrhizae form between the soil factors, which needs to be furtherly studied.

In conclusion, *C. grandiflora* Hogg. can form a good symbiotic relationship with local AM fungi after invasion. The arbuscular mycorrhizal (AM) fungi are common components in terrestrial ecosystems, it is important for the control of invasive plants to study AM fungi morphology of invasive plants and its correlation with environmental factors.

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**Table 2. Correlation analysis between AM fungi development status and soil factors in rhizosphere of *Coreopsis grandiflora*.


