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Endophytic fungi associated with the medicinal plant, *Achyranthes bidentata* Blume (Amaranthaceae)

Bing-Da Sun^{1,2}, Amanda-Juan Chen¹, Wei-Wei Gao^{1*}, Yu-Guang Zhou² and Hong-Yan Liu³

¹Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, People's Republic of China.

²Institute of Microbiology, Chinese Academy of Sciences, Beichen West Road, Chaoyang District, Beijing 100101, People's Republic of China.

³Institute of Plant Protection, Henan Academy of Agricultural Sciences, Zhengzhou 450002, People's Republic of China.

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Achyranthes bidentata is an important traditional Chinese medicinal plant, which is less infected by mycorrhizal fungi. This study reported the endophytic fungi associated with *A. bidentata* growing in five cultivation sites in China. A total of 746 isolates of endophytic fungi representing 37 fungal taxa were obtained from segments of leaves, stems and roots of this plant. Anamorphic Ascomycota were the most prevalent endophytic fungi and five yeast species and a Zygomycota species were also isolated. Endophytic colonization rate was high in both leaves (74.2%) and stems (55.6%) and the two plant parts yielded same dominant endophytic fungi of *Alternaria alternata* and *Mycosphaerella* sp. While the isolation rate of *A. alternata* was higher in leaf samples than in stem samples, the reverse was the case for *Mycosphaerella* sp. The endophytic fungal community of leaves had higher isolate richness and lower species diversity than that of stems. Only 9.4% of the root samples were infected by endophytic fungi. This is the first report of endophytic fungi associated with amaranthaceous plant in China. Environmental factors of growth stage, the adjacent vegetation and agricultural practice were thought to affect the occurrence of endophytic fungi in *A. bidentata*.

Key words: *Alternaria alternata*, *Mycosphaerella*, endophytic mycoflora, traditional Chinese medicine.

INTRODUCTION

Endophytic fungi are microbes that inhabit plant tissues at some stages in their life cycle without causing apparent harm to the host (Petrini, 1991). These fungi are now proven to exist widely within plant tissues and are rich in species diversity (Gennaro et al., 2003; Sun et al., 2008). They can play important roles in adaptation and morphogenesis of host plants and act as defenders against predators, growth promoters, and competitors of microbial pathogens or latent pathogens (Redlin and Carris, 1996; Scannerini et al., 2004). Recent comprehensive studies have revealed that endophytic

fungi were novel source of potentially useful medicinal compounds because of their copious secondary metabolites (Bhagobaty and Joshi, 2011; Kusari et al., 2012; Schutz et al., 2002; Strobel, 2003). The famous example was taxol produced by *Taxomyces andreanae* isolated from *Taxus brevifolia* (Stierle et al., 1993).

Meanwhile, unlike the many researches on other plants, less study has been focused on the endophytic fungi in plants of Amaranthaceae. Blodgett et al. (2000) studied the species composition of endophytic fungi in *Amaranthus hybridus* (Amaranthaceae) in South Africa, and reported that high percentages of endophytic fungi were isolated in leaves, petioles and roots. Plants in Amaranthaceae were thought to be less infected by mycorrhizal fungi (Peterson et al., 1985; Shanker et al., 1990), so the mutualistic effect of endophytic fungi on

*Corresponding author. E-mail: wwgao411@sina.com. Fax: 86-010-62898297.

these plants might be more important than on other plants.

Achyranthes bidentata Blume (Amaranthaceae) is a traditional Chinese medicinal plant with main efficacies of dissipating blood stasis, nourishing the liver and kidney, strengthening the bones and muscles. It is usually prescribed by practitioners of traditional Chinese medicines for the treatment of osteodynia of lumbar and knees, spasm and flaccidity of limbs (Committee of National Pharmacopoeia, 2005). Although the plant *A. bidentata* has wide distribution in the northern part of China, plant material from Jiaozuo district, Henan province (including several counties of Bo-ai, Wuzhi, Wenxian, Xiuwu etc.) was considered as good quality herb medicine because of its better medicinal efficacy. Constituent analysis of *A. bidentata* showed that the medicinal material from Jiaozuo district had higher content of medicinal ingredients such as ecdysterone, oleanolic acid and polysaccharides (Li, 2008; Zhang et al., 2001).

The endophytic fungi associated with *A. bidentata* had not been reported and so did their roles in the plants. Accordingly, the objectives of this study were to: (i) identify and quantify the endophytic fungi associated with asymptomatic *A. bidentata* plants grown in cultivated fields, (ii) determine the differences in species compositions of the endophytic fungal communities among different plant parts (leaves, stems and roots), and (iii) compare the different occurrence of endophytic fungi in the plants from different cultivation regions.

MATERIALS AND METHODS

Sample collection

All plant samples were collected from the 7th to 22th of September, 2009. The sample collection was conducted in a nursery garden (Beijing, N 40°01', E 116°16') and monoculture fields at four sites in China: Chifeng (N 42°15', E 118°57', Inner Mongolia), Anguo (N 38°23', E 115°18', Hebei province), Wuzhi (N 35°06', E 113°22', Henan province) and Wenxian (N 34°57', E 113°05', Henan Province). At each site, 20 to 30 asymptomatic plants (including leaves, stems and roots) were collected from three fields. At the laboratory, the samples were stored at 4°C and processed within 2 days of collection.

Isolation of endophytic fungi

Two leaves (including petioles) were collected randomly from each of plants and three lamina segments of 0.25 cm² (including midrib) were selected from each leaf. For stem materials, two segments with eustipes at both ends (large segment) were collected randomly from each of plants and three short segments of 0.5 cm in length were selected from each large segment. For root materials, one segment were collected from each plant and six slices of 0.2 to 0.3 cm in thickness were selected from each segment. For all the sampling sites, a total of 100 segments (or slices) of each tissue were screened for the occurrence of endophytic fungi.

Plant materials were thoroughly washed in distilled water before surface sterilization. Surface sterilization was performed by the

following immersion sequence: 75% ethanol for 1 min, NaOCl (3% available chlorine) for 3 min and 75% ethanol for 1 min (Huang et al., 2007). The samples were then dried on sterilized paper before cutting into small segments or slices. Sets of four segments (or slices) were evenly placed in each 90 mm Petri dish containing 2% malt extract agar (MEA) supplemented with chloromycetin (100 mg/L) and Rose Bengal (33 mg/L) (Photita et al., 2005). Petri dishes were sealed, incubated for 2 weeks at 25°C. The pure endophytic fungi strains were transferred to new MEA slants.

Identification of endophytic fungi

The identification of endophytic fungal strains was mainly based on the morphological characteristics of reproductive structures with the aid of several relative monographs and original descriptions of species (Barnett and Hunter, 1998; Domsch et al., 2007; Ellis, 1971; Simmons, 1995; Sutton, 1980). For those poorly sporulating isolates, the internal transcribed spacer (ITS) of ribosomal DNA were amplified and sequenced following the procedure of White et al. (1990). Similar taxon retrieved by Basic Local Alignment Search Tool (BLAST) in GenBank / NCBI was used as reference for further morphological examination and identification.

Data analysis

Colonization rate (CR) was calculated as the percentage of plant tissue segments infected by fungi. Isolation rate (IR) was determined as the number of isolates of specific fungal species obtained from plant segments divided by the total number of segments incubated. The species diversity was measured using the Shannon-Wiener index (H') and the formula was

$$H' = -\sum_{i=1}^n P_i \ln P_i \quad (\text{Pielou, 1975})$$

Where, P_i is the proportion of the i th species and n is the number of species at the site. Bray-Curtis similarity coefficient (C) was used to measure the similarity of endophytic fungal communities among five sites using the formula:

$$C = 2w / (a+b) \quad (\text{Bannister, 1968})$$

Where, w is the sum of the lesser counts of each species common to both sites and 'a and b' is the sum of all isolates obtained in each site.

The software SPSS 15.0 was used for the statistical data processing. Analyses were made of colonization rate of the endophytic fungi from plants of different regions by standard χ^2 tests. Multiple pair-wise comparisons were performed using the Table procedure of SPSS software and the Bonferroni's method was used to adjust P -values.

RESULTS

Endophytic fungi were found in 46.4% (696 out of 1500) of the plant tissues examined and 746 fungal isolates belonging to 37 species and 25 genera were obtained. Fungi were isolated most often from leaves and then followed by stems, with the average colonization rates were 74.2 and 55.6%, respectively. Endophytic fungi were isolated only in 9.4% of root slices. Colonization

Table 1. Colonization rates of *Ac. bidentata* samples from different sites.

| Plant part | Chifeng | Beijing | Anguo | Wuzhi | Wenxian |
|------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Leaves | 18 ^{a1} | 93 ^c | 98 ^c | 85 ^b | 77 ^b |
| Stems | 30 ^a | 62 ^c | 76 ^c | 46 ^b | 64 ^c |
| Roots | 0 | 26 ^a | 6 ^b | 5 ^b | 10 ^b |

¹Values within the same row followed by different letters are significantly different at the $\alpha = 0.05$ level.

Table 2. Bray-Curtis similarity coefficients (%) for all sampling sites.

| Sampling site | Beijing | Anguo | Wuzhi | Wenxian |
|---------------|---------|-------|-------|---------|
| Chifeng | 23.1 | 26.3 | 35.2 | 28.9 |
| Beijing | | 47.2 | 48.3 | 53.4 |
| Anguo | | | 65.9 | 66.7 |
| Wuzhi | | | | 80.5 |

rates of endophytic fungi in the same parts of *A. bidentata* were different among regions ($\chi^2 = 219.54$; d.f. = 4; $P < 0.001$) (Table 1). The highest colonization rate was found in samples from Beijing, while Chifeng was in the opposite instance. Samples from Anguo contained same high colonization rates with that from Beijing, except the root tissues. Samples from the two sites of Henan province, Wuzhi and Wenxian have similar colonization rates in both leaf and root tissues, while the stem samples from Wuzhi yielded lower frequency of endophytic fungi. Moreover, when all isolates obtained in samples from same site were considered as a community, high similarities were found among Anguo, Wuzhi and Wenxian (Table 2). Chifeng and Beijing had more varied endophytic fungal communities; the similarities between them and the other sites were no larger than 53.4% (Bray-Curtis similarity coefficients).

Endophytic fungal species in *A. bidentata* leaf tissues

A total of 397 endophytic fungal isolates were obtained from 371 *A. bidentata* leaf segments. These isolates were identified to 18 species: 16 species of Ascomycota and two species of basidiomycetous yeast (Table 3). Most of the identified fungi have been reported as endophytes in plants before, but *Curvularia cymbopogonis* (C.W. Dodge) J.W. Groves & Skolko was for the first time reported as plant endophytic fungi.

A. alternata (Fr.) Keissl. and *Mycosphaerella* sp. were the most frequently isolated endophytic fungi in *A. bidentata* leaf, with the average isolation rates of 58.2 and 5.8%, respectively. The isolation rates of *A. alternata* in leaf samples of different sampling sites were significantly different ($\chi^2 = 134.77$; d.f. = 4; $P < 0.001$).

Significantly higher isolation rates occurred in samples from Anguo, Wuzhi and Wenxian. *Mycosphaerella* sp. also had different isolation rates in the five sampling sites ($\chi^2 = 15.15$; d.f. = 4; $P = 0.004$). Higher isolation rates occurred in samples from Anguo, followed by Beijing, Wuzhi and Wenxian. Samples from Chifeng yielded significantly lower frequency of isolation rates of the both fungal species. In addition, *Colletotrichum gloeosporioides* sensu lato (Penz.) Penz. & Sacc. had high isolation rate in samples collected in Beijing, but was not isolated from the other four sampling sites. Other fungal species were isolated with low frequencies in *A. bidentata* leaf tissues.

Endophytic fungal species in *A. bidentata* stem tissues

In total, 301 endophytic fungal strains were obtained from 278 positive *A. bidentata* stem tissues. These isolates were identified to 25 species: 21 species of Ascomycota, one species of Zygomycota and three species of basidiomycetous yeast (Table 4). Most of these identified fungi were reported as plant endophytes on other plants except the *Mucor hiemalis* f. *corticola* (Hagem) Schipper. *A. alternata* and *Mycosphaerella* sp. were the dominant fungal species in *A. bidentata* stem tissues with an average isolation rate of 15.2 and 9.6%, respectively. There was no significant difference between the isolation rates of *A. alternata* in the five sampling sites ($\chi^2 = 6.13$; d.f. = 4; $P = 0.191$), while significantly higher frequency of *Mycosphaerella* sp. were obtained in stem samples from Anguo ($\chi^2 = 47.35$; d.f. = 3; $P < 0.001$) than the other sites. The most frequently isolated endophytic fungus in stem samples from Beijing was *C. gloeosporioides* sensu lato, and this species was not obtained from the other

Table 3. Species composition (percentages) of endophytic fungi in *Ac. bidentata* leaves.

| Fungal species / genera | Chifeng | Beijing | Anguo | Wuzhi | Wenxian | Average isolation rate (%) | Reported as plant endophytes |
|---------------------------------------|------------------|-----------------|-----------------|-----------------|-----------------|----------------------------|------------------------------|
| Ascomycota | | | | | | | |
| <i>Acremonium strictum</i> | — | 2 | — | — | — | 0.4 | Yes, several |
| <i>Alternaria alternata</i> | 10 ^{a1} | 55 ^b | 77 ^c | 80 ^c | 69 ^c | 58.2 | Yes, several |
| <i>Aspergillus niger</i> | 3 | — | — | — | — | 0.6 | Yes, Wani et al., 2010 |
| <i>Cladosporium cladosporioides</i> | — | 1 | — | — | — | 0.2 | Yes, several |
| <i>Colletotrichum capsici</i> | — | 3 | — | — | — | 0.6 | Yes, Chlebicki, 2002 |
| <i>Colletotrichum gloeosporioides</i> | — | 21 | — | — | — | 4.2 | Yes, several |
| <i>Curvularia cymbopogonis</i> | — | — | — | — | 1 | 0.2 | No, only present study |
| <i>Diaporthe phaseolorum</i> | — | 13 | 1 | — | 1 | 3.0 | Yes, Guo et al., 2000 |
| <i>Exserohilum rostratum</i> | — | — | 1 | — | — | 0.2 | Yes, Sappapan et al., 2008 |
| <i>Leptosphaeria</i> sp. | — | — | 7 | 1 | — | 1.6 | — |
| <i>Mycosphaerella</i> sp. | 1 ^a | 7 ^{bc} | 13 ^c | 4 ^b | 4 ^b | 5.8 | — |
| <i>Neofabraea</i> sp. | — | 1 | — | — | — | 0.2 | — |
| <i>Penicillium oxalicum</i> | — | — | 4 | — | — | 0.8 | Yes, Vega et al., 2006 |
| <i>Phoma pinodella</i> | — | — | 1 | — | 1 | 0.4 | Yes, Sánchez et al., 2010 |
| <i>Stemphylium</i> sp. | 4 | 1 | 1 | 3 | — | 1.8 | — |
| Unidentified Dothideomycetes | — | — | 1 | — | — | 0.2 | — |
| Basidiomycota | | | | | | | |
| <i>Cryptococcus magnus</i> | — | — | 1 | — | 1 | 0.4 | Yes, Isaeva et al., 2010 |
| <i>Pseudozyma aphidis</i> | — | 2 | — | — | 1 | 0.6 | Yes, Sláviková et al., 2009 |
| Total | 18 | 106 | 107 | 88 | 78 | 79.4 | |

¹Values within the same row followed by different letters are significantly different at the $\alpha = 0.05$ level.

four sampling sites. Other fungal species were isolated with low frequencies in *A. bidentata* stem tissues.

Although the leaves and stems contained same dominant endophytic fungal species, the isolation rates of these species were different. There was significantly more *A. alternata* in leaf samples than in stem samples ($\chi^2 = 198.979$; d.f. = 1; $P < 0.001$). Quite the contrary, there was more *Mycosphaerella* sp. in stem samples than in leaf

samples ($\chi^2 = 5.079$; d.f. = 1; $P = 0.024$). The communities of endophytic fungi from leaf and stem tissues showed different species diversities: a large number but low diversity ($H' = 1.175$) of endophytic fungal community was found in *A. bidentata* leaf tissues. Compared with leaf, there were less but more diverse ($H' = 2.527$) of endophytic fungi in stem tissues. The similarity between endophytic fungal communities of leaf and stem was 48.4% (Bary-Curtis coefficient).

Endophytic fungal species in *A. bidentata* root tissues

Endophytic fungi were found to occur with low frequencies in root tissues of *A. bidentata*, and only 48 isolates were obtained from 47 positive slices. These isolates were identified to 17 species: 14 species of Ascomycota and three species of basidiomycetous yeast (Table 5). The relatively higher frequencies were found from

Table 4. Species composition (percentages) of endophytic fungi in *Ac. bidentata* stems.

| Fungal species / genera | Chifeng | Beijing | Anguo | Wuzhi | Wenxian | Average isolation rate (%) | Reported as plant endophytes |
|---|------------------|-----------------|-----------------|-----------------|-----------------|----------------------------|--|
| Ascomycota | | | | | | | |
| <i>Acremonium implicatum</i> | — | — | — | 1 | — | 0.2 | Yes, Huang and Kelemu, 2004 |
| <i>Acremonium strictum</i> | — | — | 6 | — | 1 | 1.4 | Yes, several |
| <i>Alternaria alternata</i> | 17 ^{a1} | 10 ^a | 18 ^a | 11 ^a | 20 ^a | 15.2 | Yes, several |
| <i>Cladosporium cladosporioides</i> | — | 3 | — | 10 | 8 | 4.2 | Yes, several |
| <i>Colletotrichum capsici</i> | — | 3 | — | — | — | 0.6 | Yes, Chlebicki, 2002 |
| <i>Colletotrichum gloeosporioides</i> | — | 33 | — | — | — | 6.6 | Yes, several |
| <i>Diaporthe phaseolorum</i> | — | 5 | 5 | — | — | 2.0 | Yes, Guo et al., 2000 |
| <i>Edenia achyranthi</i> | — | — | 1 | — | — | 0.2 | No, only present study |
| <i>Exserohilum rostratum</i> | — | — | — | 1 | — | 0.2 | Yes, Sappapan et al., 2008 |
| <i>Fusarium avenacum</i> | — | — | — | — | 2 | 0.4 | Yes, Crous et al., 1995 |
| <i>Fusarium equiseti</i> | — | — | 9 | 2 | 2 | 2.6 | Yes, Maciá-Vicente et al., 2009 |
| <i>Fusarium moniliforme</i> | — | — | — | — | 1 | 0.2 | Yes, Rodrigues and Menezes, 2005 |
| <i>Leptoshaeria</i> sp. | — | — | 1 | — | 4 | 1.0 | — |
| <i>Mycosphaerella</i> sp. | — | 9 ^a | 31 ^b | 3 ^a | 5 ^a | 9.6 | — |
| <i>Myrothecium roridum</i> | — | — | — | 3 | 1 | 0.8 | Yes, Tejesvi et al., 2006 |
| <i>Penicillium oxalicum</i> | — | — | — | 4 | 4 | 1.6 | Yes, Fisher et al., 1994 |
| <i>Phoma glomerata</i> | — | 1 | 2 | — | — | 0.6 | Yes, Gangadevi and Muthumary, 2007 |
| <i>Plectosporium tabacinum</i> | — | 3 | — | — | 1 | 0.8 | Yes, D'Amico et al., 2008; Götz et al., 2006 |
| <i>Stemphylium</i> sp. | 12 | — | 2 | — | — | 2.8 | — |
| <i>Verticillium dahliae</i> | — | 3 | — | 6 | 9 | 3.6 | Yes, Götz et al., 2006 |
| Unidentified Dothideomycetes | — | — | 3 | — | — | 0.6 | — |
| Zygomycota | | | | | | | |
| <i>Mucor hiemalis</i> f. <i>corticola</i> | — | — | — | 1 | — | 0.2 | No, only present study |
| Basidiomycota | | | | | | | |
| <i>Cryptococcus aureus</i> | — | — | — | 2 | — | 0.4 | No, only present study |
| <i>Cryptococcus magnus</i> | 1 | — | 1 | — | — | 0.4 | Yes, Isaeva et al., 2010 |
| <i>Pseudozyma aphidis</i> | — | — | 3 | 8 | 9 | 4.0 | Yes, Sláviková et al., 2009 |
| Total | 30 | 70 | 82 | 52 | 67 | 60.2 | |

¹Values within the same row followed by different letters are significantly different at the $\alpha = 0.05$ level.

Verticillium dahliae Kleb. and *Plectosporium tabacinum* (J.F.H. Beyma) M.E. Palm, W. Gams & Nirenberg, and the majority of isolates were obtained in samples from Beijing. The species composition of the endophytic fungi in root tissues was very different with the community; similarity between root and leaf was 3.6% and that of between root and stem was 18.9% (Bary-Curtis coefficient).

DISCUSSION

This is the first report of endophytic fungi species in *A. bidentata*, and most of the identified species had been reported on other plants before. *Cryptococcus aureus* M. Takash. and *Mucor hiemalis* f. *corticola* (Hagem) Schipper were for the first time reported as plant endophytic fungi, although the two fungal species both occurred in low frequencies in *A. bidentata* stem tissues. No host specific endophytic fungal species was found in this plant. According to the study of Blodgett et al. (2000), endophytic fungi were isolated with high frequencies in both *A. hybridus* leaves and roots and the dominant species were *Alternaria tenuissima* group. This study indicated that *A. bidentata* yielded different dominant endophytic fungi of *Al. alternata* and that the colonization of root by endophytic fungi was low.

The identification of *Alternaria* isolates from *A. bidentata* followed the same criteria used for *A. hybridus* (Simmons, 1995). The distinguishing characteristics of these isolates were: (i) spore chains were complex and branches were long (1 to 9 conidia); (ii) beaks were rare and, if present, were short, broad and never branched. As one of the most cosmopolitan small spored *Alternaria* species, *A. alternata* occur as saprophytes on many kinds of plant and other substrates (Domsch et al., 2007). It had also been reported as an endophytic fungi in a variety of plants; for

example, low occurrences were reported in *Parthenium hysterophorus* (Asteraceae) (IR = 4%), *Triticum aestivum* (Gramineae) (IR = 4.89%), *Broussonetia papyrifera* (Moraceae) (IR = 2%) and *Ligustrum lucidum* (Oleaceae) (IR = 3%). On the other hand, high occurrences were reported in *Eucommia ulmoides* (Eucommiaceae) (IR = 39.7%), *Forsythia suspense* (Oleaceae) (IR = 63.8%), *Forsythia giraldiana* (Oleaceae) (IR = 33%), *Forsythia ovata* (Oleaceae) (IR = 37.8%), *Berberis poitrtii* (Berberidaceae) (IR = 62.6%) and *Rhus potanini* (Anacardiaceae) (IR = 23.1%) (Larran et al., 2002; Novas and Carmarãna, 2008; Romero et al., 2001; Sun et al., 2008). The pathogenic *Alternaria* species reported on *A. bidentata* was *A. achyranthi* J.Z. Zhang et T.Y. Zhang (Zhang et al., 1999), which was not obtained from healthy plants in this study.

Different endophytic fungal mycoflora were found in leaves, stems and roots in this study. Although the up-ground parts (leaf and stem) yielded the same dominant endophytic fungal species, the species diversities of the endophytic fungal communities of the two parts were different. Large number but low diversity of endophytic fungi was found in leaf tissues compared with stem tissues; foliar endophytic fungal community has relatively low diversity with obvious dominant species. This phenomenon has also been reported on other plants by various former studies (Blodgett et al., 2000; Carroll, 1995; Gond et al., 2007; Rollinger and Langenheim, 1993; Verma et al., 2007). Compared with the up-ground parts, the root tissues of *A. bidentata* yield an almost completely different endophytic mycoflora characterized by low isolation rates and different species composition. This result was different from that of *A. hybridus*; our study showed high colonization rates (CRs) in *A. bidentata* stems (55.6% on average) and extraordinarily low CRs in roots (9.4% on average), while the opposite results had been reported on *A. hybridus* in which

CRs in roots varied from 60 to 91%, and only 5 to 8% of the stem tissues were colonized by endophytic fungi (Blodgett et al., 2000). These were the only two species in Amaranthaceae that have been studied on the occurrence of endophytic fungi, and it is interesting that they showed different distribution pattern of endophytic fungi within roots and stems.

A special endophytic fungal community with high isolation rates of *Colletotrichum* spp. was found in nursery garden (Beijing), where the *A. bidentata* was surrounded by trees such as *Sophora japonica* (Fabaceae) and *Prunus persica* (Rosaceae); also, the *A. bidentata* was planted without rotation. Considering that no *Colletotrichum* isolates was obtained from samples of monoculture fields, environmental factors of adjacent vegetation and agricultural practices might be the causable reason for the occurrence of these fungi in *A. bidentata*. The genus *Colletotrichum* (teleomorph: *Glomerella*) is an important and widespread group; some species are serious plant pathogens (Jeffries et al., 1990) and some are frequent endophytic fungal colonizers of tropical plants (Rodrigues and Petrini, 1997; Cannon and Simmons, 2002). As one of the most common and widely distributed species of the genus, the species concepts of *C. gloeosporioides* is still unsettled; at least 600 taxa which show varying degrees of pathogenicity, host-specificity and genetic homogeneity were placed into the synonymy of this species (Arx, 1957; Hyde et al., 2009). Recently, polyphasic characters including morphology, physiology, pathogenicity, cultural characteristics and secondary metabolites was introduced to study *Colletotrichum* species (Cai et al., 2009). The *C. gloeosporioides* isolates from *A. bidentata* were identified by morphological characteristics and rDNA-ITS sequences and so the name *C. gloeosporioides* sensu lato was used.

Few studies have been focused on the

Table 5. Species composition (percentages) of endophytic fungi in *Ac. bidentata* roots.

| Fungal species / genera | Chifeng* | Beijing | Anguo | Wuzhi | Wenxian | Average isolation rate (%) | Reported as plant endophytes |
|-------------------------------------|----------|---------|-------|-------|---------|----------------------------|--|
| Ascomycota | | | | | | | |
| <i>Acremonium</i> sp. | — | — | — | — | 1 | 0.2 | — |
| <i>Acremonium strictum</i> | — | — | — | — | 1 | 0.2 | Yes, several |
| <i>Alternaria alternata</i> | — | — | 2 | — | — | 0.4 | Yes, several |
| <i>Aureobasidium pullulans</i> | — | 1 | — | — | — | 0.2 | Yes, Martini et al., 2009 |
| <i>Cladosporium cladosporioides</i> | — | 2 | — | — | 1 | 0.6 | Yes, several |
| <i>Fusarium oxysporum</i> | — | 1 | 1 | — | — | 0.4 | Yes, several |
| <i>Fusarium solani</i> | — | — | 1 | — | — | 0.2 | Yes, several |
| <i>Myrothecium roridum</i> | — | — | — | 1 | — | 0.2 | Yes, Tejesvi et al., 2006 |
| <i>Myrothecium verrucaria</i> | — | — | — | — | 1 | 0.2 | Yes, Nalini et al., 2005 |
| <i>Penicillium oxalicum</i> | — | — | — | 1 | — | 0.2 | Yes, Fisher et al., 1994 |
| <i>Phaeosphaeria</i> sp. | — | — | — | — | 2 | 0.4 | — |
| <i>Plectosporium tabacinum</i> | — | 7 | — | 2 | — | 1.8 | Yes, D'Amico et al., 2008; Götz et al., 2006 |
| <i>Verticillium dahliae</i> | — | 15 | — | 1 | 2 | 3.6 | Yes, Götz et al., 2006 |
| Unidentified Arthopyreniaceae | — | 1 | — | — | — | 0.2 | — |
| Basidiomycota | | | | | | | |
| <i>Cryptococcus magnus</i> | — | — | 2 | — | — | 0.4 | Yes, Isaeva et al., 2010 |
| <i>Pseudozyma aphidis</i> | — | — | — | — | 1 | 0.2 | Yes, Sláviková et al., 2009 |
| <i>Rhodotorula</i> sp. | — | — | — | — | 1 | 0.2 | — |
| Total | — | 26 | 6 | 5 | 8 | 9.6 | |

*No fungus was isolated from root slices of samples from Chifeng.

endophytic fungi in annual plants so as to know the frequencies of these fungi among different growth stages. Larran et al. (2007) compared the frequencies of endophytic fungi among five growth stages of wheat (*Triticum aestivum* L.) and found that the frequencies of endophytes in leaf and glume tissues were high at late growth stages

(milky ripe stage and ripe for cutting stage), while that in stems was high at middle growth stage (flag leaf visible stage). In this study, Chifeng, the northernmost site chosen in this study, has a growing season of 20 to 30 days earlier than that of other sites and *A. bidentata* was in the flowering stage when collected (*A. bidentata* was in

vegetative growth stage for other sampling sites). Significantly less endophytic fungi were obtained in samples there. This indicated that the frequency of endophytic fungi in *A. bidentata* could be affected by growth stage.

The sampling sites of Wenxian and Wuzhi were among the most appropriate places to plant

A. bidentata. Recently, successful introduction and cultivation of *A. bidentata* in Anguo was reported (Li et al., 2008). Compared with other places, there were more *A. alternata* in leaf tissue of plants from Anguo, Wuzhi and Wenxian. Since high similarities were found among endophytic fungal communities of the three sites, a correlation between endophytic mycoflora and the quality of herb medicine could be hypothesized. Further study on the effect of endophytic fungi in the process of medicinal ingredients synthesis in host plant is needed.

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