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The colonization of plants by dark septate endophytes (DSE) in the valley-type savanna of Yunnan, southwest China

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The colonization of 120 plant species by dark septate endopytes (DSE) in the valley-type savanna of Jiansha River, southwest China was investigated. It was found that about 84% of plants were colonized by DSE to some extent. Morphological polymorphism of DSE was observed and 8 plant families were recorded as potential hosts for DSE. High colonization rate of DSE in the savanna vegetation implies that these fungi may play an important role in the adaption of plants to the dry-hot stress environment.

Key words: Valley-type savanna, dark septate endophyte.

INTRODUCTION

Dark septate endophytes (DSE) are a group of heterogeneous root-associated endophytic fungi which are characterized by melanized intercellular and intracellular runner hyphae and so-called microsclerotia (aggregation of dark, thick-walled, closely packed inflated cells) within epidermis and cortex of plant roots (Jumpponen and Trappe, 1998; Wilson et al., 2004; Mandyam and Jumpponen, 2005a; Muthukumar et al., 2006; Silvani et al., 2008). Moreover, their existence has been found prevalent all over the world (Jumpponen and Trappe, 1998; Wang and Zhao, 2005; Šraj-Kržič et al., 2006). Although, confusions of their taxonomic affinity and obscure effects on hosts remain a problem due to little understanding of their teleomorph (most DSE rarely sporulate or remain absolutely sterile under culture conditions) and their variable impacts on hosts ranging from positive to negative in different experimental conditions (Horton et al., 1998; Jumpponen, 2001; Girlanda et al., 2002; Urcelay, 2002; Grünig et al., 2004; Korhonen et al., 2004; Addy et al., 2005). It is likely that they may have a role as

a mutualist in a degenerate ecosystems, for that they colonize in plant roots without obviously virulent effects on the hosts and even may confer benefits to the host. The proposed benefits could be facilitation of plant nutrient absorption and water uptake, protection of plants against pathogen and herbivores, and reinforcement of plant tolerance to stress conditions. (Grünig et al., 2002; Frenot et al., 2005; Mandyam and Jumpponen, 2005b; Korhonen et al., 2004). So far, most of the studies have been concerned about their omnipresence, especially their occurrence in stressful cold and drought environments (Robinson, 2001; Olsson et al., 2004; Frenot et al., 2005; Mandyam and Jumpponen, 2005a; Wu et al., 2009). Their cryptic ecological functions as mycorrhizae or compensate (or alternative) of mycorrhizae imply their latent ecological significance in these areas (Addy et al., 2005; Beauchamp et al., 2005; Schulz and Boyle, 2005; Weishampel and Bedford, 2006; Kapoor et al., 2008). But little attention has been paid to their occurrence in the savanna vegetation.

Valley-type savanna locates in the valleys of different rivers in southwest China, among which Jiansha River valley-type savanna is the most typical one. It is an endangered type of savanna around the world with its own peculiarity and diversity of vegetation (Jin and Ou,

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Sampling site	Location	AT (°C)	AP (mm)	No. of plant families	No. of plant species	No. of plant sample (w/h)
Р	102°13' to 102°57'E 25°24' to 26°22'N	15.6	963.9	28	46	46(16/30)
Х	102°47' to 103°18'E 25°47' to 26°32'N	20.2	700	12	29	29 (2/27)
Y	101°35' to 102°06'E 25°23' to 26°06'N	21.9	629	29	67	67(12/55)
Total				41	120	142(30/112)

Table 1. The natural conditions and numbers of the samples collected in the sample sites.

P, Pudu River; X, Xiao River; Y, Yuanmou; AT, annual mean temperature; AP, annual mean precipitation; w/h, numbers of woody samples/numbers of herbaceous samples.

2000). The occurrence of DSE in this special savanna vegetation was investigated and the morphological polymorphism of DSE and its ecological role in this stressful ecosystem was also discussed in the present study.

MATERIALS AND METHODS

Study sites

Dry-hot valleys in Jiansha River lie in the southwest of China $(25^{\circ}40'-28^{\circ}15'N, 100^{\circ}30'-103^{\circ}30'E)$, mainly in Yunnan and Sichuan provinces. The annual mean temperature and precipitation are 20 to 27°C and 600 to 800 mm, respectively (mainly from June to October). The evaporation is almost six times as much as its precipitation. The valley-type savanna vegetation which is composed of grasses and dwarf bushes is developing in these dry-hot valleys (Jin and Ou, 2000; Zhao, 2006; Li et al., 2007). In the present study, three valley-type savanna communities, Pudu River (P), Xiao River (X) and Yuanmou (Y) developing in Jiansha River in Yunnan province were chosen as study sites. The locations and climatic information of the study sites are shown in Table 1.

Sample collecting and processing

Plants in the sample areas were randomly collected and taken back to the lab for further processing. The numbers of plant samples collected from each site are shown in Table 2. Roots of each sample were washed under running tap water to remove contaminants and then fixed in 1/2 FAA (formalin: glacial acetic acid: 70% ethanol = 1:1:18 V/V/V, diluted twice when using).

Estimation of dark septate endophytes (DSE) colonization in plant roots

The fixed roots were taken out and cleared in 10% KOH in a water bath of temperature 90°C for 1 to 2 h. Then the root samples were processed with the method as described by Berch and Kendruch (1982). The stained roots were cut into short pieces of about 2 cm and put onto slides to examine the fungal colonization under a lightcompound microscope (Olympus BX-31) at 400x magnification. The colonization extent of hyphae (H%) and microsclerotia (MS%) was estimated according to the magnified intersection method (McGonigle et al., 1990) and the fungal morphologic characters were photographed under a light-compound microscope (Olympus BX-51).

Statistical analysis

Difference of colonization extent of DSE among three sample sites, and between woody and herbaceous plants in each site was analyzed by the package of SPSS 12.0. Data on percentage of DSE colonization were arcsine transformed to fulfill the assumption of normality and homogeneity of variances before analysis.

RESULTS

Colonization of dark septate endophytes (DSE) in plant roots

The colonization status of DSE in dry-hot valleys of Jiansha River was shown in Table 2. About 84% (119/142) of plant specimens investigated were colonized by DSE in the valley-type savanna of Jiansha River. The colonization frequency of DSE in P, X and Y were 94% (43/47), 72% (21/29) and 82% (55/67), respectively (Table 2). The colonization extent of most plants (70%) was below 30% (Table 3). There were 8 plants whose hyphal colonization extent was above 50% with the maximal colonization extent of 93% in Nouelia insignis (P) (Tables 2 and 3). Meanwhile, the occurrence of microsclerotia was only found in 52 plant samples, among which the colonization extent of 39 samples was below 5% (Tables 2 and 3). The colonization extent of microsclerotia in Laggera alata (P), Cymbopogon distans (P), Reinwardtia indica (P), Desmodium microphyllum (Y) was above 10%. The highest reached 33% in Cymbopogon distans (Tables 2 and 3).

Eight (8) plant families, Agavaceae, Amaranthacae, Coriariaceae, Gesneriaceae, Musaceae, Pteridaceae, Thymelaeaceae and Verbenaceae were recorded as DSE hosts for the first time in the present study. It was noticed that plants of two domain plant families, Asteraceae and Poaceae, were extensively colonized by DSE. The non-existence of fungal structures was found only in four samples of *Bidens bipinnata* (X), *Laggera pterodonta* (X), *Arthraxon hispidus* (Y) and *Paspalidium flavidium* (Y) in the two families (Table 2).

The mean hyphal colonization extent of herbaceous

Table 2. Colonization extent of DSE in the valley-type savanna in southwest China.

Plant	Sample site	Н%	MS%	Plant	Sample site	H%	MS%
Acanthaceae				Asteraceae			
Barleria cristata	Р	57.33	2	Xanthium sibiricum	Х	0.67	0
	Y	45.95	0		Y	5.33	0
Strobilanthes cusia	Р	22	5.33	Zinnia elegans	Y	11.33	0
Adiantaceae				Bignoniaceae			
Adintum philipense	Y	4.67	0	Incarvillea arguta	Р	2	0
Agavaceae					Y	10.67	0
Agave americana	Y	35.33	0.67	Boraginaceae			
Amaranthacae				Cynoglossum laceolatum	Y	9.88	0
Achyranthes aspera	Р	0	0	Brassicaceae			
	Y	3.75	0	Sophora davidii	Р	2.74	0
Amaranthus spinosus	Х	2	0	Cactaceae			
A. viridis	Y	0	0	Opuntia stricta	Х	15.33	0.67
Asclepiadaceae				Chenopodiaceae			
Calatrania sistentes	V	44.40	0	Chenopodium	P	0.07	0.07
Calotropis gigantea	X	11.19	0	ambrosioides	P	0.67	0.67
	Y	21.33	2		Х	0	0
Cryptolepis buchananii	Р	21.53	0		Y	0	0
Asteraceae				Convolvulaceae			
Artemisia roxburghiana	Х	39.33	2.67	lpomoea batatas	Y	28.92	1.2
Bidens bipinnata	Х	0	0	Porana racemosa	Х	12	0
	Y	28	0	Coriariaceae			
Blainvillea acmella	Y	4.67	0	Coriaria sinica	Р	17.31	0
Conyza. blinii	Y	4	0.67	Cyperaceae			
C. anadensis	Y	22	1.33	Cyperus niveus	Y	10.67	0.67
C. japonica	Х	2	0	C. rotundus	Х	54.67	0.67
Eupatorium adenophorum	Р	8.33	0		Y	21.33	0
Helianthus annuus	Y	3.33	0	Eriophorum comosum	Y	44	2
Laggera alata	Р	1.33	10.67	Equisetaceae			
	Х	34	6	Equisetum diffusum	Y	3.33	0
L. pterodonta	Х	0	0	Euphorbiaceae			
	Y	1.33	0	Acalvpha acmophvlla	Y	25.33	0.67
Nouelia insianis	Р	92.67	0.67	Elsholtzia cvpriani	Y	7.33	0
Parthenium hvsterophorus	Y	12.67	2.67	Euphorbia antiquorum	Р	21.33	0
Siegesbeckia orientalis	P	0.67	0	E. heterophylla	Ŷ	2.67	0
	X	22	0.67	E. hirta	X	11.27	0.7
	Ŷ	10	0.67		Ŷ	19.33	0.67
Sonchus oleraceus	Ŷ	8.67	0	F. rovleana	Ý	52.21	1.47
Tridax procumbens	×	0	0				
Fuphorbiaceae	X	U	Ū	Moraceae			
E thymifolia	Y	57 33	4	Ficus tikoua	Р	25 33	5 33
E. arynmona	×	0	0	Musaceae	•	20.00	0.00
Glochidion puberum	P	6 67	0	Musa nana	Y	1 33	0.67
Ricinus communis	v v	0.07	0	Musa nana Murtaceae	I	1.00	0.07
Gesneriaceae		0	0	Fucalvotus citriodo	v	Ω	0
	V	0.67	0	Nyctaginacoao	1	0	0
l abiatao		0.07	0	Roerhavia diffusa	V	10 52	1 05
Easialae Elebolizia ruguloso	D	20 67	0		I	10.55	1.05
E stachyodos	r V	20.07 1 20	0	Fravinus malaconhulla	D	56	0
L. SIDUIYUUUUS		1.39	0		F	00	U
	Ĭ	0.07	U	годседе			

Table 2. Contd.

Paraphlomis lancidentata	Х	21.33	0	Andropogon yunnanensis	Р	8.67	7.33
Rabdosia sculponeata	Р	6	0.67		Х	0.67	0
Leguminosae				Arthraxon hispidus	Х	0	0
Acacia farnesiana	Y	30	2.67	Bothriochloa pertusa	Х	20.67	9.33
Albiazia kalkora	Y	1.74	0		Y	64.67	10
Alysicarpus vaginalis	Y	10.71	0	Chloris virgata	Х	14.67	2
Atylosia scarabaeoides	Y	41.33	1.33	Cymbopogon distans	Х	34.67	4.67
Arachis hypogaea	Y	4	0		Р	0.67	32.67
Cajanus cajan	Y	39.33	0	Digitaria cruciata	Х	48	0
Crotalaria reynaudiana	Y	0	0	Heteropogon contortus	Х	4	0.67
Dalbergia yunnanensis	Р	29.33	0		Y	0.67	0.67
Desmodium microphyllum	Y	25.33	17.89	Neyraudia reynaudiana	Р	23.33	0
D. multiflorum	Х	0	0		Y	42.67	0.67
Flemingia strobilifera	Р	23.33	0	Paspalidium flavidium	Y	0	0
Glycine max	Y	0.67	0	Setaria plicata	Р	4.67	1.33
Indigofera linifolia	Х	29.33	0	Tripogon bromoides	Р	20.67	0.67
Tamarindus indica	Y	4	0		Y	37.33	6.67
Tephrosia purpurea	Y	0	0	Zea mays	Y	0.67	0
Vigna aconitifolia	Y	5.33	0	Polygonaceae			
Linaceae				Polygonum hydropiper	Х	0	0
Reinwardtia indica	Р	2.3	10.34	P. statice	Y	0	0
Malvaceae				P. urophyllum	Р	3.33	0
Abutilon indicum	Y	16	0	Pteridaceae			
Sida acuta	Y	5.33	0	Onychium lucidum	Р	18	0
S. szechuensis	Р	4	0	Pteris vittata	Р	2	0
	Х	2.31	0	Rhamnaceae			
	Y	0	0	Ziziphus mauritiana	Y	4.67	0
Urena procumbens	Р	0	0	-	Р	62.67	6.67
Rosaceae				Thymelaeaceae			
Agrimonia nepalensis	Р	8	2	Wikstroemia canescens	Р	18	0
Spiraea martinii	Р	40	0	Ulmaceae			
Rubhceae				Trema angustifolia	Р	16	0
Emmenopterys henryi	Р	14	0	Urticaceae			
Rubia cordifolia	Р	22	0.67	Debregeasia edulis	Р	0	0
Rutaceae				Pouzolzia sanguinea	Р	4	0
Boenninghausenia	Р	2 67	1 22	Varbanaaaa			
sessilicarpa	Г	2.07	1.55	Verbenaceae			
Sapindaceae				Verbena officinalis	Р	8	2
Dodonaea anfustifolia	Y	14	2	Vitex negundo	Р	15.87	0
Selaginellaceae					Y	18	0
Selaginella davidii	Р	12	0	Zygophyllaceae			
S. pulvinata	Y	24	2	Tribulus terretris	Y	0	0
S. sinensis	Y	30	6				
Solanaceae							
Capsicum annuum	Y	0	0				
Datura stramonium	Y	0	0				
Solanum indicum	Р	8	0				
S. verbascifolium	Р	20.67	0				

P, Pudu River; X, Xiao River; Y, Yuanmou; H%, colonization extent of hyphae in root samples; MS%, colonization extent of microsclerotia in root samples.

Colonization extent of	NC in sampling sites			Colonization extent of	NC in sampling sites		
hyphae	Р	Х	Y	microsclerotia	Р	Х	Y
0	3	8	12	0	29	19	42
0 to 30% (≤30%)	38	16	45	0 to 5% (≤5%)	10	8	21
30 to 50% (≤ 50%)	1	4	7	5 to 10% (≤10%)	4	2	3
50 to (>50%)	4	1	3	10 to (>10%)	3	0	1
Max	92.67%	54.67%	64.67%	Max	32.67%	9.33%	17.89%

Table 3. Colonization extent of hyphae and microsclerotia in the different sites.

P, Pudu River; X, Xiao River; Y, Yuanmou; NC, Numbers of plant samples being colonized.

Table 4. Hyphal colonization extent of woody and herbaceous plants in the different sites

Sampling	wo	ody	herba	ceous	woody+herbaceous		
site	Mch (%)	Mcm (%)	Mch (%)	Mcm (%)	Mch (%)	Mcm (%)	
Р	26.9±6.3	1.2±0.5	9.7±1.6	2.5±1.1	15.7±0.7	2.0±0.8	
Х	6.8±4.4	0.0±0.0	13.6±3.2	1.0±0.4	13.2±3.0	1.0±0.4	
Y	11.7±3.9	0.5±0.3	14.6±2.3	1.2±0.4	14.0 ± 2.0	1.0±0.3	
P+X+Y	19.4±3.9	0.8±0.3	13.0±1.4	1.5±0.4	14.4±1.4	1.4±0.3	

P: Pudu River; X: Xiao River; Y: Yuanmou; Mch (%): mean colonization extent of hyphe in root samples ± SE; Mcm (%): mean colonization extent of microsclerotia in root samples ± SE.

plant roots of the dry-hot valleys in Jiansha River was 13%, lower than that of woody plants (20%). For each site alone, the colonization extent of herbaceous plants was higher than that of the woody plants in X and Y, but the situation was contradictory in P. The mean hyphal colonization extent of woody plants in P was 27%, which was the highest in the three sites (Table 4).

According to analysis of variance (ANOVA), there was no difference of H% and MS% among three sites (pH%=0.398>0.05, pMS%= 0.586>0.05). Moreover, the colonization extent between woody and herbaceous in each site showed rare difference. Significant difference of hyphal colonization between woody and herbaceous plants was only found in P (pH%=0.013<0.05).

Morphologic observation of dark septate endophytes (DSE) in roots

The characterized darkly melanized, septate hyphae and microsclerotia formed by tightly packed swollen cells with thick-walls were the most common and distinguished anatomic structures in roots under microscope (Figure Relatively. slightly pigmented hvphae 1A). and microsclerotia were also observed (Figure 1E). Meanwhile, some special structures were noticed, including hyphal coils around the inner wall of the cells (Figure 1B), the spore-like swells of hyphae (Figure 1F), brain-like microsclerotia (Figure 1H), mycelial labyrinth formed by a great deal of hyphae spreading over the roots (Figure 1G), hyaline microsclerotia-like structures packing in the cell (Figure 1D), and strands of spores-like cells (Figure 1C).

DISCUSSION

Occurrence of dark septate endophytes (DSE) in dryhot environments

Ubiquity of DSE in arid environments was demonstrated in previous studies. DSE have been reported to form symbiotic association with oak trees and Carex species, respectively in the Whetstone Savanna in Jackson County, Oregon and a prairie savanna community in northeastern Illinois (Miller et al., 1999; Valentine et al., 2002). The abundant and consistent colonization of dominant shrubs and grasses in arid southwestern USA rangelands by DSE was reported by Barrow and Aaltonen (2001). Barrow further illustrated the extensive colonization of DSE in this area in 2003 and 2004 (Barrow, 2003, 2004). In a recent study of mycorrhizal and dark septate endophytic fungi under the canopies of desert plants in Mu Us Sandy Land of China, Wu et al. (2009) indicated a good establishment of DSE in the desert ecosystem, in which all the 20 dominant plant species examined were colonized by DSE.

Colonization of plants belonging to Asteraceae and Poaceae by DSE was frequently observed (Väre et al., 1992; Cázares et al., 2005; Menoyo et al., 2007). Newsham et al. (2009) reviewed that DSE were often recorded to associate with plant species of the two



Figure 1. Morphological polymorphism of DSE in plant roots of dry-hot valleys in Jiansha River. A, Dark hyphae and microsclerotia in *Zizyphus yunnanensis*; B, hyphal coils in *Euphorbia thymifolium*; C, strands of spore-like cells in *Tripogon filiformis*; D, hyaline microsclerotia-like structures in *Ipomoea batatas*; E, slightly colored hyphae and microsclerotia in *Laggera alata*; F, spore-like swells of hyphae in *Conyza Canadensis*; G, mycelial labyrinth in *Euphorbia royleana*; H, brain-like microslerotia in *Laggera alata*; Bar, 50 µm.

families in Arctic and sub-Arctic ecosystems. Prevalence of DSE in plants of Asteraceae in the Chaco Serrano Woodland from central Argentina was documented by Fracchia et al. (2009). Among the 21 plants of Asteraceae they collected, there were only two noncolonized plants. Thus, plants of these two families could be common hosts of DSE as shown in our study. Moreover, plants in the families of Agavaceae, Amaranthacae, Coriariaceae, Gesneriaceae, Musaceae, Pteridaceae, Thymelaeaceae and Verbenaceae, which were not included in the host range of DSE listed by Jumpponen and Trappe (1998), were also colonized by DSE in the present study. This result suggested that the host ranges of DSE and their distribution might be far beyond our knowledge.

DSE has been assumed to form mutualistic association with plants in arid ecosystems (Addy et al., 2005), as they might improve plant fitness through their direct participation in water uptake of plant roots or promotion of plant performance in the ecosystems with threat of aridity (Mandyam and Jumpponen, 2005a). Our results were consistent with several previous field data from arid stress. In the present study, above 80% of plants in the valley-type savanna of Jiansha River were colonized by DSE, which suggested that DSE might form mutualistic association with plants in arid ecosystems (Addy et al., 2005). Otherwise, their occurrence in some other arid areas might also suggest the good adaption of DSE and their positive influence on water absorption of plants (Mandyam and Jumpponen, 2005a). This is a good argument for the point of view that DSE may have beneficial impacts on plants in arid ecosystems.

It was supposed that the difference of microenvironment in three sites might lead to differentiation of fungal colonization, but no significant difference was detected in the present study. Additionally, discrepancy of colonization extent between woody and herbaceous plants was found only in hyphal colonization extent in P. This might shed light on the non-specificity of host of DSE, as they have been found widely colonized in woody and herbaceous plants (Grünig et al., 2002; Addy et al., 2005; Schulz and Boyle, 2005).

Polymorphism of dark septate endophytes (DSE)

DSE were easily distinguished from traditional mycorrhizal fungi for their non-stained darkly melanized hyphae running inter- or intracellularly in root epidermis and cortex (Mandyam and Jumpponen, 2005a; Silvani et al., 2008). Microsclerotia with dark colors spread in the epidemic and cortical cells and were variable in size and shape. However, morphologic variations in squashed roots have been described by many researchers (Barrow and Aaltonen, 2001; Yu et al., 2001; Barrow, 2003). Light-color or even hyaline hyphae and microsclerotia which were poorly stained were simultaneously seen with the darkly melanized along hyphae and

microsclerotia (Haselwandter and Read, 1982). This might illustrate that the formation and deposition of melanin in fugal cell walls was a consistent gradual course and could be considered as a mechanism of protection of fungi from harsh environments, such as droughty in our study, as melanin was hypothesized to enhance the rigidity of fungal cell walls as well as fungal resistance against irradiation, high temperature and desiccation (Butler and Day, 1998; Robinson, 2001; Vrålstad et al., 2002; Survanarayanan et al., 2004; Addy et al., 2005; Zhan et al., 2011). It was guite possible that the pigmentation of hyphae accelerated when the environmental stress intensified, as researchers speculated that melanized fungal structures became dominant when the activity of host decreased (Barrow and Aaltonen, 2001; Barrow, 2003). The microsclerotia may be available propagula of the fungi in resistance to stress (Barrow and Aaltonen, 2001; Yu et al., 2001; Wu et al., 2009). And the hvaline hvphae may be physically active in interaction with host roots as sites of resource exchange (Barrow and Aaltonen, 2001). Moreover, some brain-like microsclerotia sliahtly different from microsclerotia usually reported in relevant studies were observed (Horton et al., 1998; Barrow and Aaltonen, 2001; Girlanda et al., 2002; Rains et al., 2003). There was no exact elucidation of significance of these hyphal dysmorphosis in their lifecycles in plant roots, but the polymorphism of DSE might to some extent involved in the enhancement of fungal adaptation in xeric ecosystem and implied the complicated interactions between fungi and the host plants.

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