International Scholars Journals

International Journal of Medicinal Plants Research ISSN 2169-303X Vol. 13 (2), pp. 001-006, February, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Influence of different *Orchidaceae mycorrhizal fungi* on the development of dendrobium candidum and D. nobile

Yang Dan, Zhi-xia Meng and Shun-Xing Guo*

Institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, 100193, China.

Accepted 10 November, 2023

***Dendrobium candidum* and *D. nobile*, rare medicinal herbs in China, are difficult in artificial propagation due to symbiotic cultivation using endophytic fungi. In order to establish a symbiotic system, we investigated several decades of endophytic fungi from Orchidaceae plants in growth promotion of dendrobe (*D. candidum* and *D. nobile*). Forty strains were inoculated upon protocorms and plantlets of dendrobes and incubated for 60 days. Symbiotic strains were screened out by survival of hosts, and the survival hosts were measured in height and weight at interval days. Six of the 40 strains were best for development of the hosts, including Strains DC-13 (*Mycena dendrobii*), AR-13 (*Mycena anoectochila*), AR-15 (*Epulorhiza* sp.), DC-8 (*Epulorhiza* sp.), DN-3 (*Gliocladium* sp.), and AR-7 (*Cephalosporium* sp.). The former three were better than the rest ones. *Gliocladium* sp. (DN-3) was first found in promotion of Orchidaceae hosts and *Cephalosporium* sp. (AR-7) was the first species of Hyphomycetes for the development of Orchidaceae hosts. Several symbiotic cultivation systems have been established, which are the basis for propagation of *Dendrobium* plants in agriculture.**

Key words: *Dendrobium candidum*, *Dendrobium nobile*, mycorrhizal fungi, Orchidaceae.

INTRODUCTION

Symbiotic relationship of endophytic fungi and Orchidaceae plants is maintained throughout the life cycle of host (Jin et al., 2009). As far 50 genera of endophytic fungi belonging to 16 families out of four phylums such as Deuteromycotina, Basidiomycota, Ascomycota and Zygomycota have been reported: most of them are species of Agonomycetaceae, Moniliaceae, Tremellomycetidae, and Agaricomycetidae, while a few in Ascomycota and Zygomycota (Zhu et al., 2004). Diversity and composition of endophytic fungal communities have been largely investigated (Feuerherdta et al., 2005; Tao et al., 2008; Hu et al., 2010), however a few studies concerned about successful symbiotic culture of fungi species and hosts (Yu et al., 2010), which is a premise for establishment of symbiosis system for bio-interaction research as well as propagation of Orchidaceae plants in agriculture.

Dendrobium candidum Wall*. ex Lindl.* and *D. nobile* Lindl. (Orchidaceae) are botanical resources of *Dendrobii Caulis* which is a traditional Chinese herb treating thirst due to impairment of *yin* or deficiency of body fluid, loss of appetite with nausea, and fever in deficiency condition after a severe disease, and impaired vision in China (Pharmacopoeia Committee of P. R. China, 2010). They are rare and endangered plants which are propagated artificially in agriculture with poor yields due to microbial contamination upon plantlets and low development of plantlets (Yu et al., 2010). Based upon symbiotic relationship of endophytic fungi and Orchidaceae plants, we have isolated from medicinal plants (Orchidacea) decades of mycorrhizal fungi strains which we have

*Corresponding author. E-mail: sxguo2006@yahoo.com.cn. Tel: +86-10-6282 9619. Fax: +86-10-6289 5120.

studied in seed germination and plantlet development for decade years (Guo and Xu, 1991; Guo et al., 1996). Here, we investigated growth of *D. candidum* and *D. nobile* incubated with 40 strains of mycorrhizal fungi of which the best six were focused in the growth-promotion of the hosts.

MATERIALS AND METHODS

Fungi strains

Mycorrhizal fungi strains (Table 1) were identified by Prof. Shun-xing Guo of the Institute of Medicinal Plant Development (IMPLAD), the Chinese Academy of Medical Sciences, China (Guo and Xu, 1990; Guo et al., 1997; Guo and Fan, 1999; Fan and Guo, 1998). They were kept in slant test-tubes at 4°C.

Plant materials

Protocorm and plantlets were obtained from seed germination of *D. candidum* and *D. nobile*. Seeds were purchased from Yunnan province, and identified by Prof. Xin-rong Chen of IMPLAD, China. Following culture activation of those strains, they were transplanted into PDA plates and incubated for 13-15 days. And then we made fungal slices upon plates with different strains by Φ8 mm cork borers.

A protocorm of *D. candidum* of an area of 1 cm^2 was placed into the center of a 100 ml flask with ½ MS media and inc ubated for 12 d at 25°C in a 12 h light:12 h dark cycle provided by 2000-Lx white fluorescent tubes. At that moment, a fungal slice was put 1 cm far from the protocorm in the flask. Triplet repeated for each strain. We observed the effects of mycorrhizal fungi stains on the growth of protocorms after 45 days of the co-cultivation of fungi and host protocorms. The protocorms free of fungi were control. '–' means death of protocorms or plantlets; '0' means no effects on growth of protocorm or plantlets; '+' means positive effects.

D. candidum plantlets in number of three to six were placed into the center of a 100 ml flask with ½ MS media. They w ere incubated for 10 d at 25°C in a 12 h light : 12-h dark cycle p rovided by 2000-Lx white fluorescent tubes, and then a fungal slice was put 1 cm far from the plantlets in the flask. Triplet repeated for each strain. After 50 days of the co-cultivation we measured height and weight of the plantlets, so did for *D. nobile* plantlets. Plantlets of the two host species free of strains were control, respectively.

The six best stains in growth promotion were co-cultivated with plantlets of *D. candidium* and *D. nobile* in the way as same as the above, respectively. Quintic repeated for each strain. Plantlet heights were determined after 15, 25, 40, 50, 60 days, respectively, while plantlet heights were measured after 60 days.

Increment % = (treatment – control) / control×100

Data analysis

Plantlet height and weight were tested by analysis of variance (ANOVA) and Tukey's honestly significant difference (P=0.05).

RESULTS

Growth of protocorms of *D. candidum*

After 45 days of co-cultivation, protocorms and plantlets most died of fungi strains -- the hypha covered over the protocorms -- which indicated most of fungi strains were the parasites of *D. candidum* (Table 1). However, Strains DC-13, CS-1, and AR-15 had no effects on *D. candidum* protocorms: the protocorms developed as same as control.

Growth of plantlets of *D. candidum* and *D. nobile*

Twenty two of all strains caused the death of plantlets of *D. candidum*, accounting for 55% of the total strains; 13 strains had no effects, accounting for 32.5%; only 5 strains such as Strains DC-8, DC-13, DN-3, AR-7, and AR-13 showed positive effects on growth of plantlets of *D. candidum*, taking up 12.5%. Eighteen strains led to the death of plantlets of *D. nobile*, accounting for 45% of the total strains; 16 strains had no effect, taking up 40%; only six strains including Strains DC-8, DC-13, DN-3, AR-7, AR-13, and AR-15, had positive effects on the growth of the plantlets of *D. nobile*, accounting for 15%.

Dynamic growth of *D. candidum* and *D. nobile* plantlets inoculated with the six fungi strains

The six fungi strains showed positive effects on the growth of plantlets of *D. candidum* (Table 2). Compared with control, plantlets of *D. candidum* had significant growth after 15 days of co-cultivation. The height increments were highest on the 50^{th} day of co-cultivation except the plantlets with Strain AR-13, and then they all decreased on the 60^{th} day that was the harvest day. Of the six strains, Strains DC-13 and AR-13 were the best for height increment (114.29% both). Table 2 also showed that height increment was not in proportion to weight increment in plantlets at the same moment, e.g., the weight increment was highest in the plantlets with Strain AR-13 (90.48%) on the 60^{th} day of co-cultivation instead of Strain DC-13 (84.13%), though the later (101.61%) was higher than the former (96.77%) in height increment. And the strains both were the best for weight increment. During the co-cultivation, *D. candidum* with Strain AR-13 had robust plantlets with green leaflets and stems. In addition, the plantlets inoculated with the six strains had inflated internodes, while the control didn't have. All the above showed that Strain AR-13 was the best of the six strains for the growth of *D. candidum* plantlets.

The six strains all promoted the growth of *D. nobile* plantlets (Table 3). And *D. nobile* plantlets vitiated similarly with *D. candidum* ones in both growth of height and weight. However, Strain AR-15 was the best for the growth of *D. nobile* plantlets (65.33% height increment and 108.75% weight increment on the harvest day).

DISCUSSION

Screening of endophytic fungi species in establishment of symbiotic relationship

Endophytic fungi of Orchidaceae plants have been

Table 1. Growth of D. candidum and D. nobile inoculated with 40 mycorrhizal fungi strains.

Host plants	Strain nos.	Fungi species	Protocorm of *D. candidum*	Plantlets of *D. candidum*	Plantlets of *D. nobile*
Aerides	AM-06	*Moniliopsis* sp.	-	-	-
Multiflorum	AM-10	*Fusidium* sp.	-	-	-
Dendrobium	DC-3	*Epulorhiza* sp.	-	0	0
Candidum	DC-6	*Cephalosporium* sp.	-	0	0
	DC-7	*Cephalosporium* sp.	-	-	0
	DC-8	*Epulorhiza* sp.	-	+	+
	DC-10	*Unidentified*	-	-	-
	DC-11	*Chromosporium* sp.	-	0	0
	DC-13	*Mycena dendrobii*	0	+	+
D. nobile	DN-1	*Rhizotonia* sp.	-	0	0
	DN-2	*Rhizotonia* sp.	-	0	0
	DN-3	*Gliocladium* sp.	-	+	+
	DN-6	*Mycena sp.*	-	0	0
	DN-7	*Gliocladium* sp.	-	0	0
	DN-10	*Moniliopsis* sp.	-	0	0
	DN-11	*Ceratorhiza* sp.	-	0	0
Bulbophyllum affine	BA-5	*Epulorhiza albertaensis*	-	-	-
	BA-10	*Chromosporium* sp.	-	-	-
Vanda teres	VT-4	*Fusoma* sp.	-	-	-
	VT-5	*Ceratorhiza goodyerae-repentis*	-	-	0
Cymbidium sinense	CS-1	*Mycena orchidicola*	0	0	0
	CS-2	*Verticillum albo-atrum*	-	-	-
	CS-4	*Mycelia sterilia*	-	-	-
Dendrobium densiflorum	DD-4	*Fusarium solani*	-	-	-
	DD-6	*Moniliopsis* sp.	-	-	-
D. gibsonii	DG-6	*Cylindrocarpon* sp.	-	-	-

Table 1. Contd.

Phaius tankervillae	PT-4	*Fusarium* sp.	-	-	-
	PT-8	*Acremonium* sp.	-	-	-
Coelogyne leucantha	CL-2	*Dactylella* sp.	-	-	-
	CL-7	unidentified	-	-	-
Anoectochilus roxburgii	AR-5	*Rhizotonia* sp.	-	-	0
	AR-7	*Cephalosporium* sp.	-	+	+
	AR-9	*Moniliopsis* sp.	-	-	-
	AR-10	*Gliocladium sp.*	-	-	-
	AR-11	*Gliocladium sp.*	-	0	0
	AR-12	*Ceratorhiza sp.*	-	-	-
	AR-13	*Mycena anoectochila*	-	+	+
	AR-15	*Epulorhiza* sp.	0	0	+
	AR-18	*Epulorhiza* sp.	-	0	0
	AR-20	*Moniliopsis* sp.	-	-	-

"-": death of hosts; "0": no effects on hosts; "+" : positive effects on hosts.

Table 2. Growth of *D. candidum* plantlets incubated with 6 fungi strains.

Strain no.	15 d		25 d		40 d		50 d		60 d		60 d	
	Height (cm)	Increment (%)	Height (cm)	Increment (%)	Height (cm)	Increment (%)	Height (cm)	Increment (%)	Height (cm)	Increment (%)	Weight (cm)	Increment (%)
DC-8	0.20±0.07^{A}	33.33	0.48±0.08^{A}	65.52	0.77±0.09^{ABC}	87.8	1.01±0.11^{A}	106.12	1.20±0.11^{A}	93.55	0.90±0.07^{B}	42.86
DC-13	0.16±0.06^{A}	6.67	0.44±0.07^{AB}	51.72	0.84±0.09^{AB}	104.88	1.05±0.10^{A}	114.29	1.25±0.08^{A}	101.61	1.16±0.11^{A}	84.13
DN-3	0.18±0.08^{A}	20	0.35±0.11^{AB}	20.69	0.64±0.13^{BC}	56.1	0.80±0.15^{B}	63.27	0.89±0.16^{B}	43.55	1.02±0.12^{AB}	61.9
AR-7	0.20±0.07^{A}	33.33	0.36±0.09^{AB}	24.14	0.60±0.14^{CD}	46.34	0.80±0.15^{B}	63.27	0.91±0.16^{B}	46.77	0.87±0.12^{B}	38.1
AR-13	0.15±0.06^{A}	0	0.48±0.09^{A}	65.52	0.88±0.10^{A}	114.63	1.05±0.11^{A}	114.29	1.22±0.13^{A}	96.77	1.20±0.10^{A}	90.48
AR-15	0.15±0.06^{A}	0	0.30±0.09^{B}	3.45	0.57±0.10^{CD}	39.02	0.83±0.12^{B}	69.39	0.95±0.10^{B}	53.23	0.93±0.09^{B}	47.62
CONTROL	0.15±0.06^{A}		0.29±0.07^{B}		0.41±0.08^{D}		0.49±0.08^{C}		0.62±0.09^{C}		0.63±0.07^{C}	

Unlike letters in a column showed significant differences at $P<0.05$.

largely studied in isolation and species identification, which is the foundation for elucidating symbiotic relationship (Hu, 2010;

Table 3. Growth of *D. nobile* plantlets incubated with 6 fungi strains.

Strain no.	15 d		25 d		40 d		50 d		60 d		60 d	
	Height (cm)	Increment (%)	Height (cm)	Increment (%)	Height (cm)	Increment (%)	Height (cm)	Increment (%)	Height (cm)	Increment (%)	Weight (g)	Increment (%)
DC-8	0.18±0.06A	12.5	0.30±0.09B	-6.25	0.59±0.13BC	43.9	0.72±0.13BCD	44	0.88±0.14BC	17.33	0.98±0.11C	22.5
DC-13	0.18±0.08A	12.5	0.34±0.09B	6.25	0.52±0.11BC	26.83	0.93±0.09AB	86	1.21±0.11A	61.33	1.50 ±0.11A	87.5
DN-3	0.20±0.07A	25	0.31±0.08B	-3.13	0.40±0.13C	-2.44	0.64±0.14CD	28	1.06±0.20AB	41.33	1.04±0.16BC	30
AR-7	0.17±0.06A	6.25	0.46±0.08AB	43.75	0.71±0.08AB	73.17	0.80±0.12BC	60	0.88±0.12BC	17.33	0.93±0.14C	16.25
AR-13	0.16±0.06A	0	0.51±0.08A	59.38	0.70±0.08AB	70.73	0.89±0.11AB	78	1.14±0.13AB	52	1.25±0.13B	56.25
AR-15	0.23±0.06A	43.75	0.55±0.09A	71.88	0.83±0.10A	102.44	1.10±0.09A	120	1.24±0.14A	65.33	1.67±0.09A	108.75
CONTROL	0.16±0.06A		0.32±0.07B		0.41±0.08C		0.50±0.10D		0.75±0.11C		0.80±0.09C	

Unlike letters in a column showed significant differences at $P<0.05$.

Feuerherdta et al., 2005; Tao et al., 2008). However, all fungi isolated or identified can't be replanted upon host successfully. Thus, it is indispensable to know them in growth promotion. Of the 40 fungi strains, Strains DC-13 (*Mycena dendrobii*), AR-13 (*Mycena anoectochila*), AR-15 (*Epulorhiza* sp.), DC-8 (*Epulorhiza* sp.), DN-3 (*Gliocladium* sp.), and AR-7 (*Cephalosporium sp.*) are symbiotic fungi. The former three were best for dendrobe plantlet development. *Epulorhiza* sp. has been proved symbiotic fungi of Orchidaceae hosts (Jin et al., 2009). Neither *Gliocladium* sp. nor *Cephalosporium* sp. have yet been found growth promotion in Orchidaceae plants until our survey was carried out. In addition, *Cephalosporium*, a genus of Hyphomycetes, gave rise to a phylum new in Orchidaceae host development (Zhu et al., 2004). Previous studies (Guo and Wang, 2001) showed that *Mycena* spp. had positive effects on seed germination of Orchidaceae plants. Here, however, we proved its roles in development of plantlets and protocorms of dendrobe.

Increasing number of endophytic fungi of Orchidaceae plants is being isolated and identified, which leads to a large task to prove their symbiotic property. We carried out this study by symbiotic culture upon tissue plantlets which are not a time-saving method. Therefore we are seeking a quick method based upon DNA co-relationships between mycorrhizal fungi and hosts.

Mechanism of symbiotic relationship

Recent studies showed that secondary metabolites of endophytic microorganisms largely contribute to seed germination and host develop-ment. Indole-3-acetic acid (IAA) produced by bacterial partners promoted seed germination of *D. moschatum* (Tsavkelova et al., 2007). Zhang et al. (1999) detected IAA in *M. dendrobii* and *M. anoectochila* which were the best strains for development of *D. candidum* and *D. nobile* in our study. gibberellic acid (GA) and naphthalene-3-acetic acid (NAA) promoted elongation of stem and root of *D. huoshanense*. We proposed that IAA, GA, and NAA were the bioactive agents in development of plantlets of *D. candidum* and *D. nobile*, which was the common prerequisite of fungi producer in symbiotic culture. Thus, we are going to determine those chemicals in other fungi strains and their roles in growth promotion of dendrobe.

ACKNOWLEDGEMENTS

This research was financially supported by the National Natural Science Foundation of China (No.30770068), the National High Technology Research and Development Program of China (No. 2008AA09Z405), and the International Science and Technology Cooperation Projects of China (No. 2009DFA32250).

REFERENCES

Fan L, Guo SX (1998). The development of orchid mycorrhizal fungi research. Microbiology, 25: 227-230.

Feuerherdta L, Petitb S, Jusaitisc M (2005). Distribution of mycorrhizal fungus associated with the endangered pinklipped spider orchid (*Arachnorchis* (syn. *Caladenia*) *behrii*) at Warren Conservation Park in South Australia. New Zealand J. Bot., 43: 367-371.

Guo SX, Fan L, Cao WQ, Xu JT (1997). *Mycena anoectochila* sp.nov. isolated from mycorrhizal roots of *Anoectochilus roxburghii* (*Orchidaceae*) from Xishungbanna of China. Mycologia, 89: 952-954.
Guo SX, Fan L (1999). *Mycena dendrobii,* a new mycorrhizal fungus. Mycosystema, 18:141-144.
Guo SX, Xu JT (1991). Studies on the effects of fungi on the course of seed germination of *Dendrobium lohohens* and *D. candidum* (Orchidaceae). Acta Acad. Med. Sin., 13: 46-49.
Guo SX, Xu JT, Cao WQ (1996). Studies on the preparation process and germination of white *Dendrobiuml* (*Dendrobium candidum*) artificial seeds. Chin. Tradit. Herb. Drugs, 27: 105-107.
Guo SX, Xu JT (1990). Isolation and culture of fungi promoting seed germination of Shihu etc medicinal plants of orchid family (*Orchidaceae).* Chin. Tradit. Herb. Drugs, 21: 30-31.
Guo SX, Wang QY (2001). Character and action of good strain on stimulating seed germination of *Gastrodia elata*. Mycosystema, 20(3): 408-412.
Hu KQ, Hou XQ, Guo SX (2010). Distribution of endophytic fungi in *Dendrobium officinale*. Microbiol. China, 37(1): 37-42.
Jin H, Xv ZX, Chen JH, Han SF, Ge S, Luo YB (2009). Interaction between tissue-cultured seedlings of *Dendrobium officinale* and mycorrhizal fungus (*Epulorhiza* sp.) during symbiotic culture. Chin. J. Plant Ecol., 33: 433-441.
Pharmacopeia Committee of P. R. China (2010). Pharmacopoeia of People's Republic of China. China Medical Science and Technology Press, Beijing.
Tao G, Liu ZY, Hyde KD, Liu XZ, Yu ZN (2008). Whole rDNA analysis reveals novel and endophytic fungi in Bletilla ochracea (Orchidaceae). Fung. Diver., 33: 101-122.
Tsavkelova EA, Cherdyntseva TA, Klimova SY, Shestakov AI, Botina SG, Netrusov AI (2007). Orchid-associated bacteria produce indole-3-acetic acid, promote seed germination, and increase their microbial yield in response to exogenous auxin. Arch. Microbiol., 188: 655-64.
Yu J, Zhao KP, Dong F, Cheng LM, Xiao SQ, Qiu DM, Hu XL (2010). Seperation and growth-promoting study of Endophyte in wild *Dendrobium candidum*. Modern Agric. Sci. Tech., 9: 96-97.
Zhang JH, Wang CL, Guo SX, Chen JM, Xiao PG (1999). Studies on the plant hormones produced by 5 species of endophytic fungi isolated from medicinal plants (Orchidacea). Acta Acad. Med. Sin., 21: 460-464.
Zhu GS, Liu ZY, Mao TF (2004). Research advances of orchid mycorrhizal fungi. Guizhou Agric. Sci., 32: 79-81.