

Low-specific Symbiotic Germination of *Dendrobium Huoshanense in Vitro*, an Endangered Epiphytic Orchid Endemic From South China

Yan-Jing Tang

Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medicinal Plant Development

Jun Dai

Wanxi College: West Anhui University

Yang Li

CAMS PUMC IMPLAD: Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medicinal Plant Development

Dong-Yu Zhou

Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medicinal Plant Development

Yong-Mei Xing

Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medicinal Plant Development

Shun-Xing Guo

CAMS PUMC IMPLAD: Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medicinal Plant Development

Juan Chen (✉ kibchenjuan@126.com)

Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medicinal Plant Development

<https://orcid.org/0000-0002-5407-8239>

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Abstract

Background: *Dendrobium huoshanense* is an endangered epiphytic orchid and is endemic to Anhui province of China with a narrow distribution. In nature, orchid seed germination depends on specific mycorrhizal fungi and it is generally assumed that there is a high specific symbiotic germination in narrow distribution orchid. To evaluate the mycorrhizal compatibility and germination-promoting activity of mycorrhizal fungi of *D. huoshanense* in vitro, we isolated and identified cultured mycorrhizal fungi from the roots of *D. huoshanense* and *D. officinale*. A total of 20 mycorrhizal fungal strains (*D. huoshanense* (n = 6), *D. officinale* (n = 12), *D. nobile* (n = 1), *D. moniliforme* (n = 1)) were used to test the germination-promoting activity of *D. huoshanense*.

Results: In symbiotic germination tests, the mycorrhizal fungi displayed different efficiency in stimulating *D. huoshanense* seed germination, nine strains stimulated seed development to an advanced seedling stage, while the rest promoted only initial seed germination to stage 2 or stage 3, and the most effective in promoting rapid seed germination was strain 12825(seb) isolated from *D. moniliforme*, besides, the second most effective fungus was strain WX-7(seb) isolated from *D. officinale*, both of them belong to *Sebacina* isolated from non-*D. huoshanense*. In this study, all twenty fungal strains formed pelotons inside seeds of *D. huoshanense* based on the trypan blue staining results.

Conclusions: Despite its narrow specific habitat preference, *D. huoshanense* was found to be a mycorrhizal generalist during symbiotic seed germination in vitro. The study is the first to demonstrate the different abilities of orchid mycorrhizal fungi to promote seed germination, protocorm formation and seedling development of *D. huoshanense* in vitro, providing important microbiological resources for its conservation and reintroduction in nature.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Tables

Table 1. Description of different seed germination stages of symbiotic in vitro culture of *Dendrobium huoshanense*. Adapted from Zi (2014).

Stage	Description
0	No germination, testa intact
1	Embryo imbibe, swollen and covered by testa
2	Embryo continue imbibe and ruptured the testa (germination)
3	Appearance of protomeristem (protocorm formation)
4	Emergence the first leaf (early stage of seedling development)
5	Emergence the second leaf and further development

Table 2. List of isolates fungal strains identified using molecular techniques.

Fungal isolates	Host orchid	Sample site	ITS sequence length (bp)	Close relatives	Accession number	Max identity (%)	Source
M-4	<i>Dendrobium huoshanense</i>	Lvan city Anhui Province	689	<i>Tulasnella cumulopuntioides</i>	LC175324	98.21	<i>Spiranthes sinensis</i> *
M-5	<i>D. huoshanense</i>	Lvan city Anhui Province	521	Uncultured Tulasnellaceae	KX387593	99.62	Orchidaceae sp.*
M-8	<i>D. huoshanense</i>	Lvan city Anhui Province	551	<i>Tulasnella sp.</i>	KF537644	99.82	<i>Liparis japonica</i> *
M-11	<i>D. huoshanense</i>	Lvan city Anhui Province	501	Uncultured Tulasnellaceae	KX387593	99.40	Orchidaceae sp.*
A-27	<i>Dendrobium officinale</i>	Anlong city Guizhou Province	680	Uncultured Tulasnellaceae	JX024734	98.82	<i>Dactylorhiza sp.</i> *
B-19	<i>D. officinale</i>	Anlong city Guizhou Province	636	Uncultured mycorrhizal fungus	AB506843	99.84	<i>Cymbidium sp.</i> *
C-27	<i>D. officinale</i>	Simao city Yunnan Province	637	Uncultured <i>Tulasnella</i>	HM230649	99.06	Direct submit
E-10	<i>D. officinale</i>	Jinhua city Zhejiang Province	638	Uncultured Tulasnellaceae	KX587479	98.12	<i>Dendrobium sp.</i> *
NC-1	<i>D. officinale</i>	Nancang city Jiangxi Province	629	Uncultured Tulasnellaceae	KM211335	99.83	Platanthera chapmanii *
XSBN-8	<i>D. officinale</i>	Jinghong city Yunnan Province	435	<i>Tulasnella sp.</i>	MN173015	100	<i>Dendrobium moniliforme</i> *
XSBN-16	<i>D. officinale</i>	Jinghong city Yunnan Province	651	<i>Tulasnella cumulopuntioides</i>	LC175326	98.43	<i>Spiranthes sinensis</i> *
WX-15	<i>D. officinale</i>	Lvan city	467	<i>Tulasnella sp.</i>	MT611039	99.36	Orchidaceae sp.*

		Anhui Province					
S6	<i>Dendrobium nobile</i>	Jinghong city	593	<i>Tulasnella sp.</i>	JN863900	98.86	<i>Dendrobium nobile*</i>
		Yunnan Province					
M-3	<i>D. huoshanense</i>	Lvan city	700	Uncultured Serendipitaceae	MG707400	96.17	<i>Pleione sp.*</i>
		Anhui Province					
M-9	<i>D. huoshanense</i>	Lvan city	396	Sebacinales sp.	MN918490	100	Direct submit
		Anhui Province					
A-26	<i>D. officinale</i>	Anlong city	571	Sebacinales sp.	MN918489	98.42	Direct submit
		Guizhou Province					
B-24	<i>D. officinale</i>	Anlong city	898	Uncultured Serendipitaceae	MG707427	98.08	<i>Pleione*</i>
		Guizhou Province					
WX-1	<i>D. officinale</i>	Lvan city	431	Sebacinales sp.	MN918489	97.68	Direct submit
		Anhui Province					
WX-7	<i>D. officinale</i>	Lvan city	635	Sebacinales sp.	MN918489	98.71	Direct submit
		Anhui Province					
12825	<i>D. nobile</i>	Jinghong city	441	Uncultured Serendipitaceae	MN918489	98.41	Direct submit
		Yunnan Province					

“*” showed Orchidaceae species marked.

Figures



Figure 1

Samples map of *Dendrobium huoshanense* and *D. officinale* collected in this study. a: Luan city, Anhui Province; b: Nanjing city, Jiangsu Province; c: Jiaxing city, Zhejiang Province; d: Jinhua city, Zhejiang Province; e: Nanchang city, Jiangxi Province; f: Guangzhou city, Guangdong Province; g: Anlong city, Guizhou Province; h: Simao city, Yunnan Province; i: Jinghong city, Yunnan Province; Samples of *D. huoshanense* was collected at site a, Samples of *D. officinale* were collected at site a-i. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

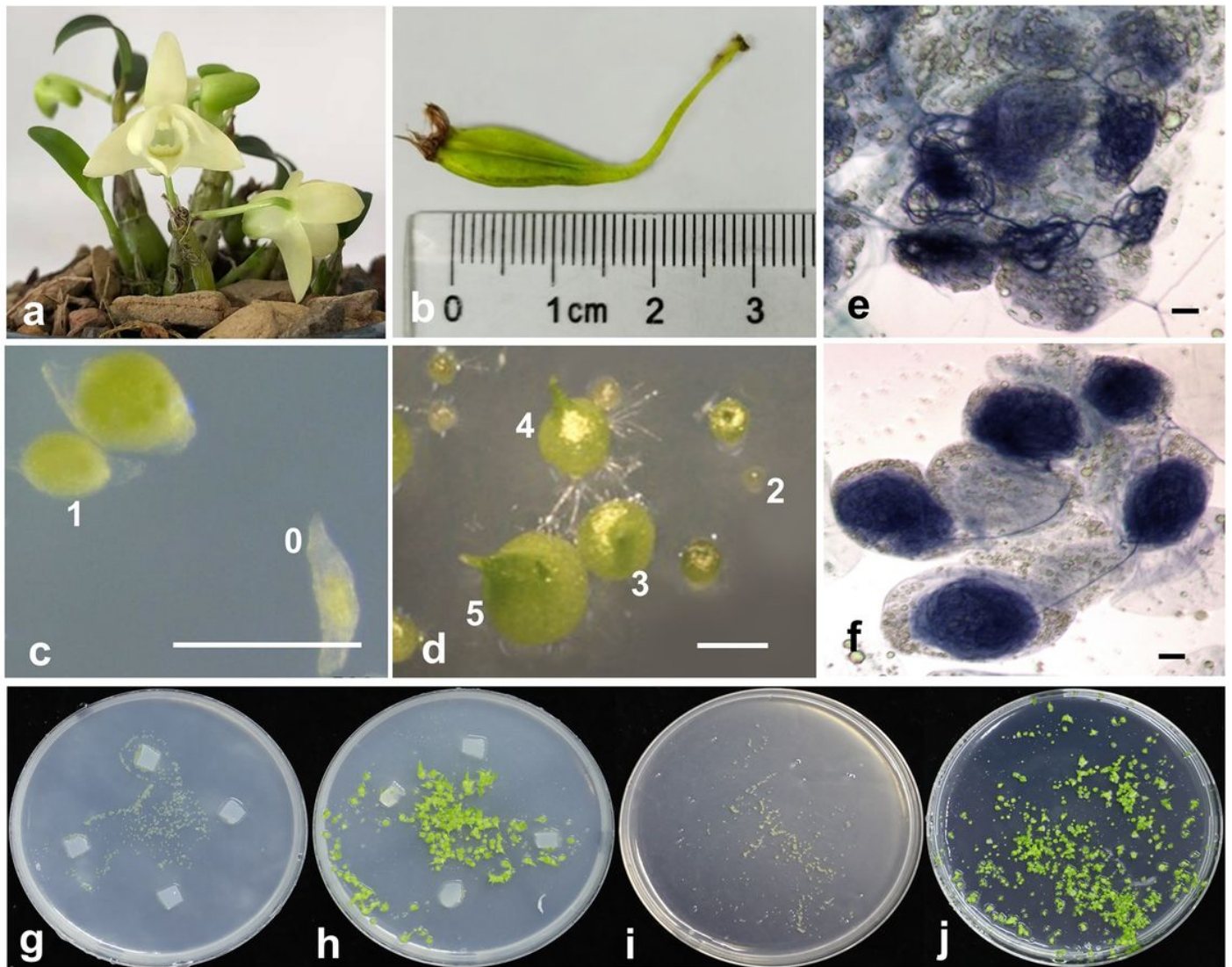


Figure 2

Overall growth form and different stages of the seed developmental stages of *Dendrobium huoshanense*. a: Flower of *D. huoshanense*; b: Capsule of *D. huoshanense*; c-d: Symbiotic seed germination of *D. huoshanense* (stage 0-5); e: Trypan blue staining showing partial colonization of protocorm cells after incubation with starin B-19; f: Trypan blue staining showing majority colonization of protocorm cells after incubation with starin 12825; g-h: Symbiotic seed germination with fungi B-19(tul) and 12825(seb) respectively at 12 weeks after sowing seeds; i-j: Asymbiotic seed germination on OMA medium (control 1) and $\frac{1}{2}$ MS medium (control 2) respectively at 12 weeks after sowing seeds; Scale bar = 500 μ m (except in "e-f" = 20 μ m)

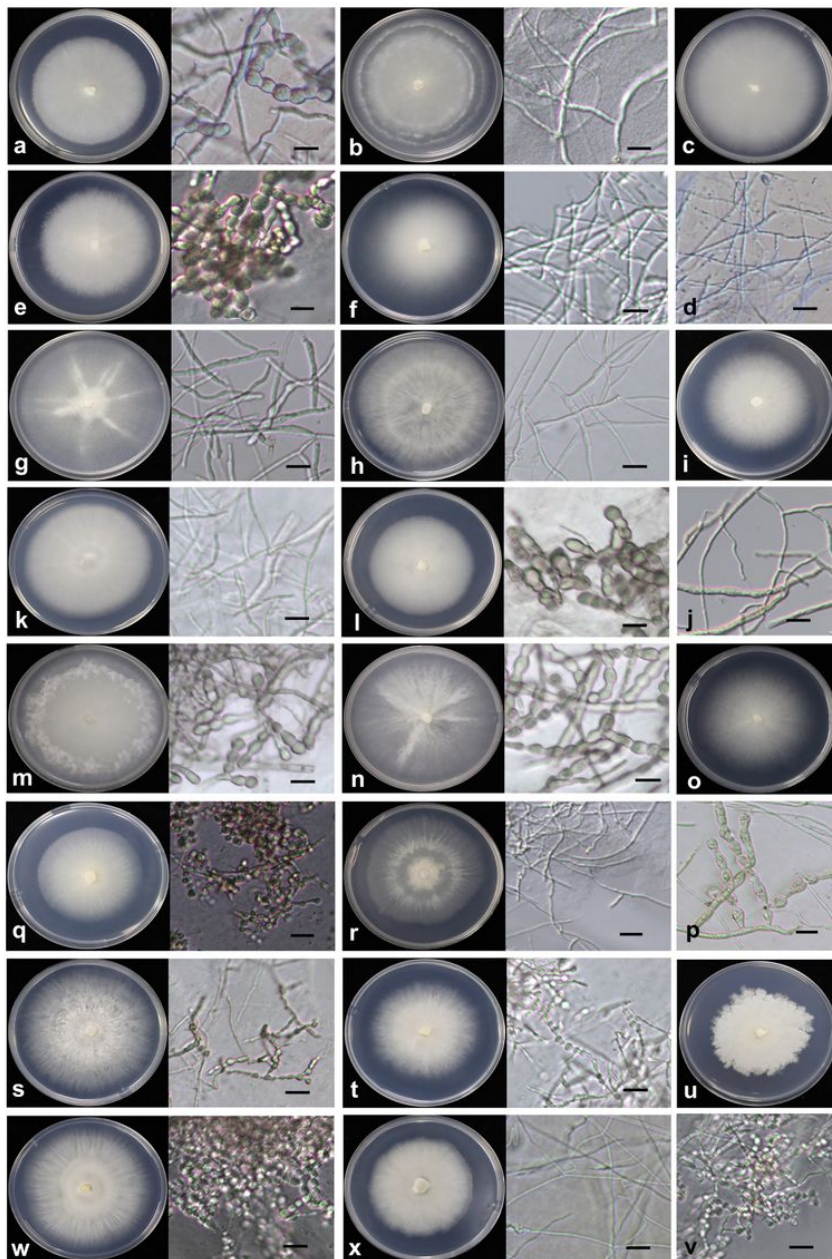


Figure 3

Morphological characteristics of isolated fungal strains in our study. Tulasnellaceae numbers: a: M-4, b: M-5, c: M-8, e: M-11, f: WX-15, g: A-27, h: B-19, i: C-27, k: E-10, l: NC-1, m: XSBN-8, n: XSBN-16, o: S6; Sebaciniales numbers: q: M-3, r: M-9, s: WX-1, t: WX-7, u: A-26, w: B-24, x: 12825; d, j, p, v represent M-8, C-27, S6, A-26 morphological characteristics respectively. Scale bar = 20 μ m

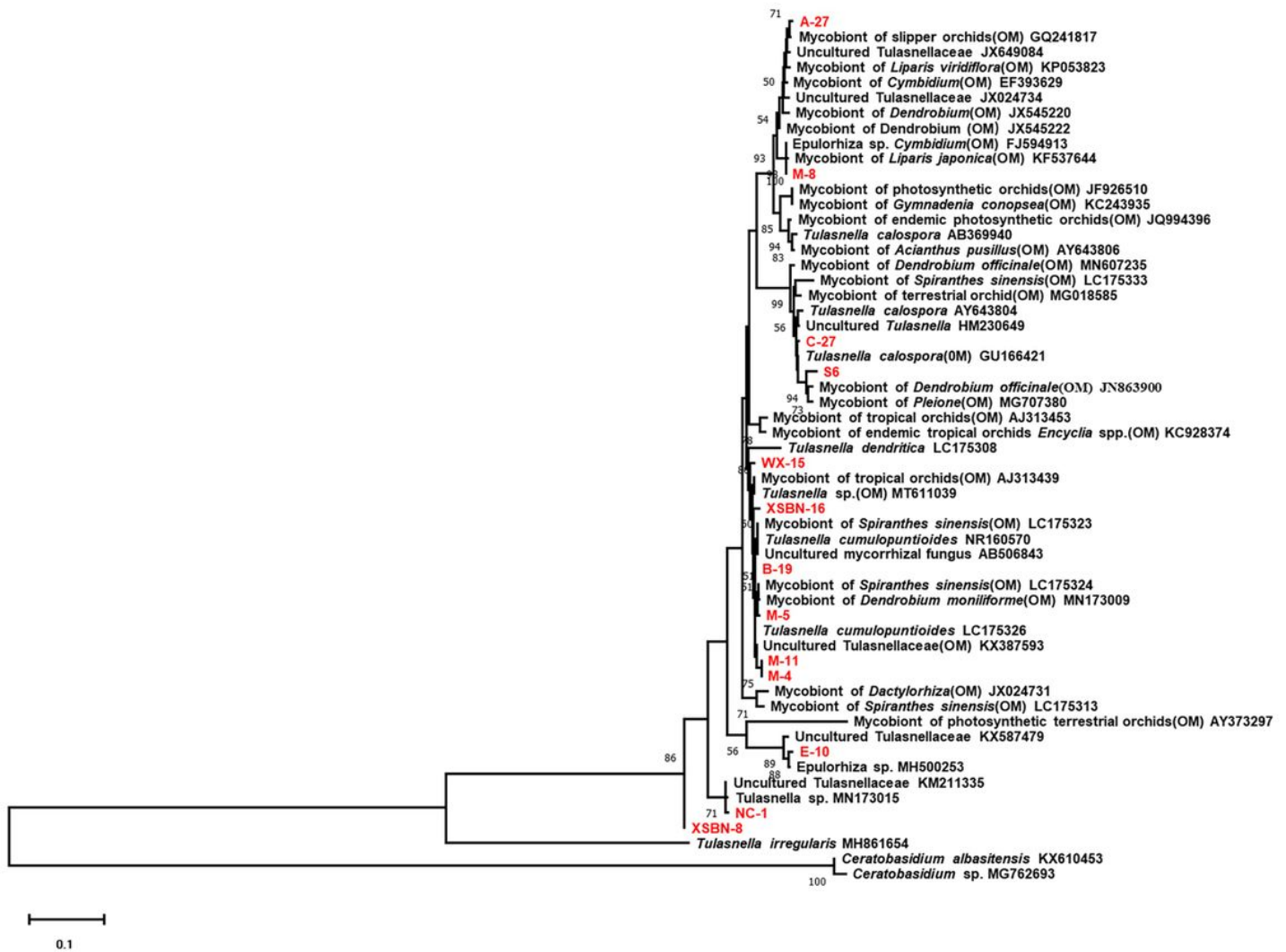


Figure 4

Phylogenetic relationships of the mycorrhizal fungi used in this study (in red) based on ITS rDNA sequences by neighbor-joining analysis. Bootstrap values > 50% are shown at branches from 1000 replicates. Two *Ceratobasidium* sp. served as an outgroup in *Tulasnella* phylogenetic tree.

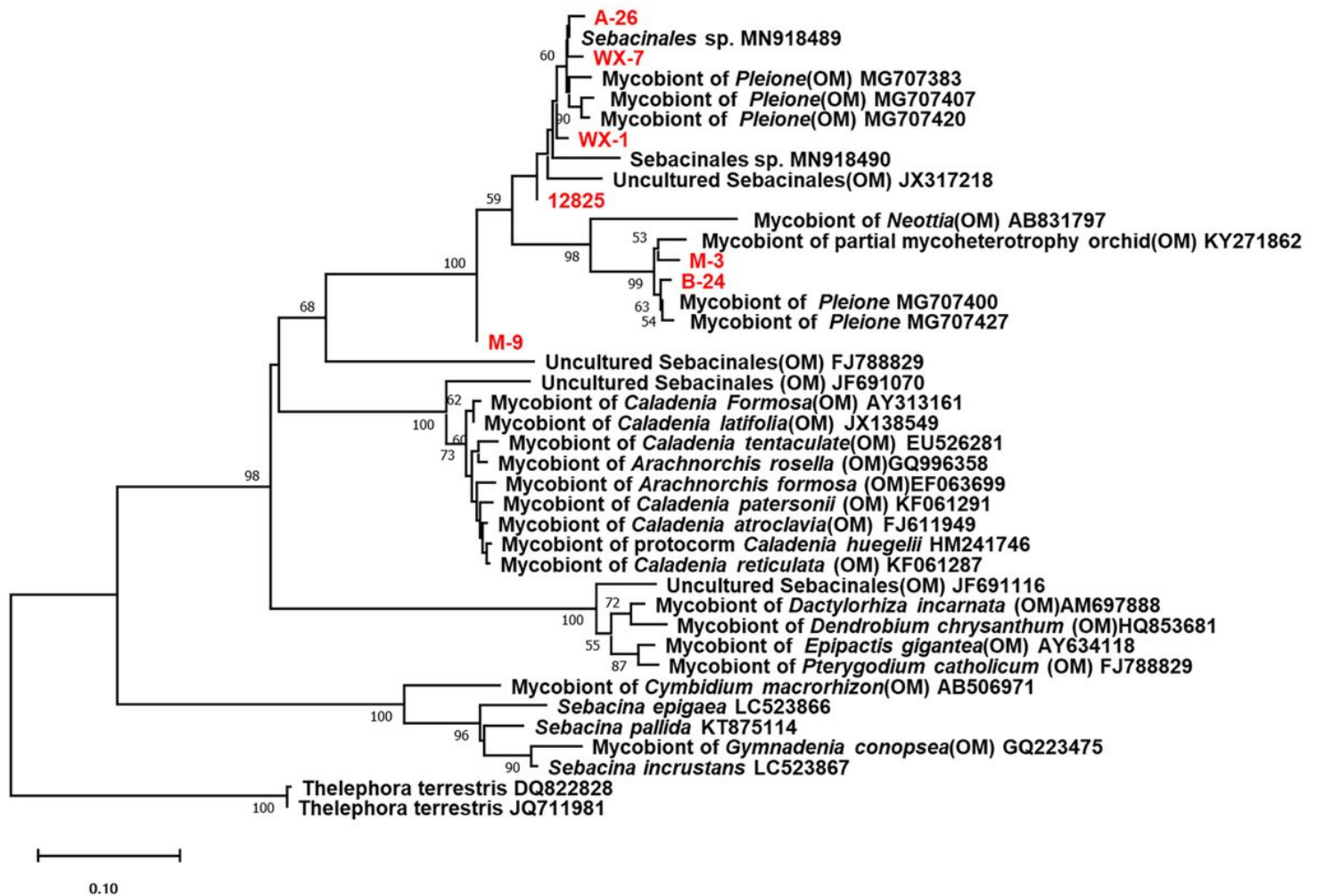


Figure 5

Phylogenetic relationships of the mycorrhizal fungi used in this study (in red) based on ITS rDNA sequences by neighbor-joining analysis. Bootstrap values > 50% are shown at branches from 1000 replicates. Two *Thelephora terrestris* served as an outgroup in *Sebacina* phylogenetic tree.

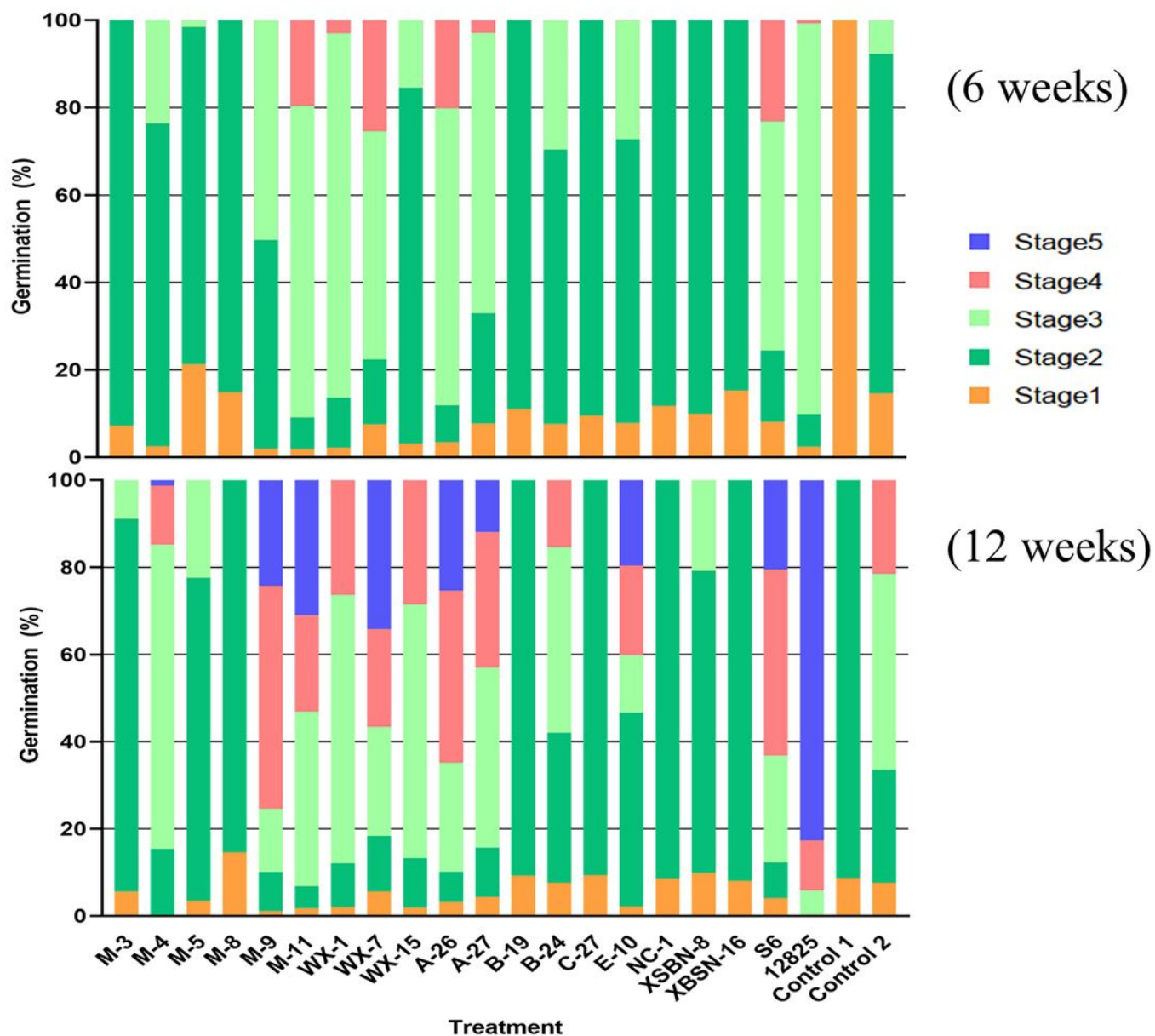


Figure 6

Differences in germination rate of *D. huoshanense* seeds incubated with each fungal isolates after 6 and 12 weeks of symbiotic culture and asymbiotic culture.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTableS2.docx](#)
- [SupplementaryTableS1.docx](#)