



**Full Length Article**

## ***Castanea henryi* Roots Serve as Host for *Ganoderma lucidum***

Huan Xiong<sup>1</sup>, Joseph Masabni<sup>2</sup>, Feng Zou<sup>1\*</sup> and Deyi Yuan<sup>1\*</sup>

<sup>1</sup>Key Laboratory of Cultivation and Protection for Non-Wood Forest Trees, Ministry of Education, Central South University of Forestry and Technology, Changsha 410004, Hunan, China

<sup>2</sup>Texas A&M AgriLife Extension, Texas A&M University System, 1710 FM 3053 N, Overton, TX 75684, USA

\*For correspondence: zoufeng06@126.com; csuftyuanyi@126.com

### **Abstract**

A new fungal strain was isolated from a fruiting body of *Ganoderma* Karst. grown in Chongyi county, Jiangxi Province, China. Morphological characterization and ITS rDNA sequence analysis suggested that the strain belongs to *Ganoderma lucidum* Karst., and we designated it as strain G18. Methods for culturing G18 and *Castanea henryi* (Skan) Rehd. Et Wils cuttings were established, and the ability of *C. henryi* cuttings serve as host for *G. lucidum* was studied *in vitro*. Ten days after inoculation, G18 grew freely on the living roots of *C. henryi* and the co-cultured mycelium formed fruiting body primordium, primitive stalk and primitive cap compared to free living mycelia. In inoculated cutting roots, transmission electron microscopy (TEM) showed that hyphae colonized the epidermal and cortical cells which retained their intact cell wall, cytoplasm, and organelles. There was no cell disintegration observed, and no hyphae were found in the apoplastic spaces. No mature fruiting bodies were observed in co-cultured and free-living mycelium at the end of the trial. TEM also showed that co-cultured roots had obvious cytoclasis. Our results indicated that G18 could inoculate living *C. henryi* roots and live endophytically in early stages, and *C. henryi* could promote G18 differentiation into the primordium stage of fruiting body development. © 2019 Friends Science Publishers

**Keywords:** Fungi; Fruiting body development; Cuttings; Saprophytic; Parasitic

### **Introduction**

*Ganoderma* Karst. is a genus of Basidiomycota including both saprophytic and parasitic fungi, and is distributed worldwide (Lloyd *et al.*, 2018a; Tchoumi *et al.*, 2018). To date, 250 *Ganoderma* species have been described all over the world (Zhou, 2017). *Ganoderma lucidum* is a species of *Ganoderma* with medicinal value. It was first recorded approximately two thousand years ago in 'The Shen Nong Herbal', a classical textbook of oriental medical science, and is now recognized by the Therapeutic Compendium and the American Herbal Pharmacopoeia (Sanodiya *et al.*, 2009; Zhang *et al.*, 2017). In recent years, more attention has been paid to its medicinal and biotechnological effects, since species of the genus *Ganoderma* can act as biofactories for producing pharmacologically active secondary metabolites such as bioactive triterpenoids, polysaccharides, oligosaccharides, and ganoderic-acid (Shiao, 2003; Hajjaj *et al.*, 2005; Shi *et al.*, 2010; Kues *et al.*, 2015; Zhang *et al.*, 2017; 2018a,b), and act as producers of ligninolytic enzymes applicable in numerous processes (Dias *et al.*, 2010; Liu *et al.*, 2012; Čilerdžić *et al.*, 2016). To meet market and research demands, artificial cultivation of *Ganoderma* has gradually spread from China, Japan, and the United States to all over the world (Zhou, 2017). All

cultivation methods are based on its saprophytic lifestyle. Though cultivation methods have improved constantly, almost all growers now prefer to adopt wood-log cultivation or substitute cultivation to produce fruiting bodies of *G. lucidum*. Production of fruiting bodies involves many steps, including tree harvest, preparation and sterilization of substrate, bagging and sterilization, spawn development and embedding in substrate, and transfer into mushroom house for maturation (Zhou, 2017). However, *Ganoderma* species are primarily white rot fungi found on dead trees and on live trees (Lloyd *et al.*, 2018b; Xing *et al.*, 2018) which indicates that we can grow *G. lucidum* using live trees based on the parasitic lifestyle of the fungus.

A study of the collections of laccate species of *Ganoderma* in the U.S. showed that the most frequent host (68%) is hardwood. Among hardwoods, *Castanopsis fargesii*, *C. sclerophylla*, and *C. carlesii* of Fagaceae family are the most suitable species for *Ganoderma* cultivation (Lloyd *et al.*, 2018a). *Castanea henryi* is an important non-wood species of the *Castanea* genus in the Fagaceae family and Fagales order. *C. henryi* is used for timber and starch production and is widely distributed in southern China with one million hectares of cultivated area (Yang *et al.*, 2013; Fan *et al.*, 2017; Xiong *et al.*, 2018a, b). This hardwood species has

been increasingly used as a woody grain since the nut has several favorable characteristics, including a high starch content of 47.58–56.94% (Zheng *et al.*, 2002), a high mineral nutrition content, and 18 amino acids (Fan *et al.*, 2015). Additionally, successful cultivation of *Dictyophora echinovolvata*, an edible fungus belonging to family Phallaceae has been reported in *C. henryi* forest land. This new practice of agroforestry is considered a sustainable form of land management that optimizes the use of natural resources (Santiago-Freijanes *et al.*, 2018; Wu *et al.*, 2018; Yao *et al.*, 2018). However, other studies have shown that some *Ganoderma* species could also act as a pathogen causing root and butt-rot disease, for they were regularly seen attached to the base of dying trees (Taylor, 1969; Wood and Ginns, 2006; Tchoumi *et al.*, 2018).

In order to provide a higher biomass production per unit of land and save the cost of artificial cultivation, rooted cuttings of *C. henryi* were used as the host to cultivate *G. lucidum in vitro*. Thus, the goal of this research is to study the feasibility of a new cultivation model of *G. lucidum*. The major objectives were 1)- to determine whether *G. lucidum* could inoculate living *C. henryi* roots and 2)- to investigate the relationship between *G. lucidum* and *C. henryi* when co-cultured.

## Materials and Methods

### Isolation of the Fungi

Fruiting bodies were collected from roots of *Castanopsis chunii* Cheng tree in Shiluo Forestry Station, a natural preserve of broad-leaved trees in Chongyi county, Jiangxi Province, China. At an altitude of 480 m, the thickness of soil humus was 2–3 cm. *Castanopsis* and *Choerospondias* (30–40 years old) were the dominant tree species, and *Castanea*, *Schima*, and *Quercus* as updated species, with a canopy density between 0.6 and 0.7. Collected fruiting bodies were placed in plastic bags and stored at 4°C until later use of fungal isolation.

Fungi were isolated from fruiting bodies by direct plating. The collected sample was soaked in cool tap water and washed gently to remove excess soil. The sample surface was sterilized with 75% ethanol for 10 s, and then rinsed four times in sterile distilled water. Using an inoculation loop, small pieces of fruiting bodies were collected from the inside of the stalk, plated on potato dextrose agar (PDA; 200 g/l potato, 20 g/l glucose, 3 g/l KH<sub>2</sub>PO<sub>4</sub>, 1.5 g/l MgSO<sub>4</sub> · 7H<sub>2</sub>O; 7 g/l agar), and cultured in the dark at 28°C for several days (Chen *et al.*, 2017). Plates were observed periodically and selected filamentous fungal colonies were re-inoculated at least five times to remove contaminants or undesirable colonies.

### Morphological Identification

Morphological characteristics including colony diameter,

color, thickness, texture, and pigmentation, and the colony color on the reverse side were examined after the fungus was cultured on PDA for 7 days in the dark at 28°C.

### Molecular Identification

The internal transcribed spacer (ITS) region was amplified using the ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') primers with thermocycling conditions of an initial step of 95°C for 3 min followed with 35 cycles of 95°C for 40 s, 54°C for 45 s, and 72°C for 1 min, and a last step of 72°C for 10 min. PCR products were purified and sequenced at Majorbio co. (Shanghai, China), using forward and reverse PCR primers.

### Phylogenetic Trees

The G18 sequence was submitted to the NCBI database and sequences of most closely matching the G18 sequence were selected using the Basic Local Alignment Search Tool (BLAST). Sequences including G18 and closely-related fungi from NCBI database were analyzed by neighbor joining method using distances from Kimura's two-parameter model with the MEGA X.0 software system which performed 1,000 bootstrap replicates to assess support for nodes (Zhang and Yao, 2019).

### Plant Material and Growth Conditions

Seeds of *Castanea henryi* cv. Huali 1 were collected from a chinquapin experimental field at Central South Forestry University of Science and Technology in Rucheng county, Chenzhou city, Hunan province (25°33'N, 113°45'E). Seeds were surface sterilized, and part of the cotyledons were excised before seed embryos were placed on aseptic MS (Murashige and Skoog, 1962) medium in glass culture tubes under white fluorescent lamps (50 mol/m<sup>2</sup> s) with a photoperiod/dark period of 14/10 h at a temperature of 25 ± 2°C (Xiong *et al.*, 2018b). The culture conditions (light and temperature) were kept constant for all the following described experiments. Three weeks later, seedlings of truncated hypocotyls were then transferred to MS medium containing 1.5 mg/l indole butyric acid for four weeks to induce adventitious roots (Xiong *et al.*, 2018b).

### Plant/Fungus Co-cultures

Rooted cuttings were transferred to conical flasks containing solidified agar (12 g/l agar) of low-carbon medium (PAD/4 + MS/4) and a pH 5.8. Mycelium of G18, cultured on PDA for 1 week, was collected using a sterile 5-mm cork borer. Two or three mycelium disks per flask were used to inoculate roots of chinquapin cuttings. Ten flasks were inoculated with G18, ten were used as control without inoculation (free living cuttings), and ten were used as control without cuttings (free living mycelium). The

lower parts of all flasks were covered with silver paper to protect the roots from light. The experiment was arranged as a randomized completed block design with ten replications (one plate is one replication).

### Microscopic Observation

In order to ascertain the relationship between G18 and *C. henryi*, root segments surrounding the mycelium were fixed in 2.5% (w/v) glutaraldehyde in phosphate-buffered saline (PBS; pH 7) overnight at 4°C. Using the paraffin sectioning method, cut sections were observed using an optical microscope (BX-51, Olympus, Tokyo, Japan) and transmission electron microscopy (TEM; Hitachi TEM System 7700, Japan) (Gao et al., 2018).

## Results

### Morphological Characteristic

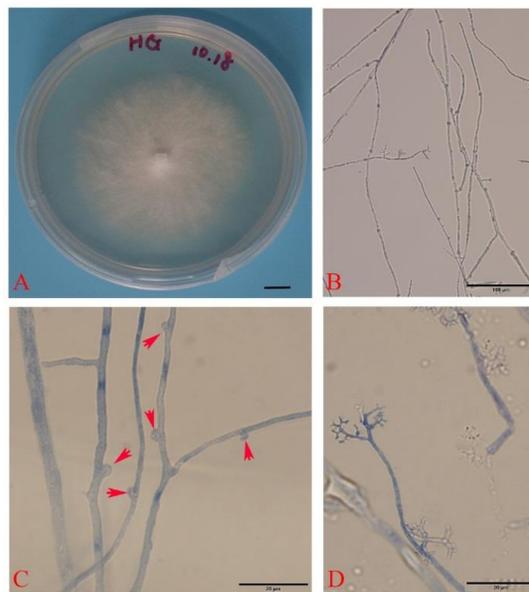
G18 formed a thin white colony after incubation in the dark at 28°C for 7 days on PDA medium. Colony diameter ranged from 45 to 50 mm (Fig. 1A). Generative hyphae were colorless, thin-walled, with lots of clamp connections, occasionally branched, and 2–4 μm in diameter (Fig. 1B and 1C). Hyphae tips with treelike branches were viewed under the microscope (Fig. 1B and 1D).

### Molecular Characteristics

Molecular analysis of G18 resulted in a 599 bp ITS rDNA sequence (Fig. 2). The ITS rDNA sequence was compared to available sequences obtained by BLAST from the GenBank database. The neighbor-joining phylogenetic tree showed that the sequence has 99.9% identity to the sequence of *G. lucidum* strain P2 and strain C6, which is supported by a bootstrap of 100% (Fig. 3).

### Development of the Fungi when Co-cultured with Cuttings

After 10 days of co-culture, roots of inoculated *C. henryi* cuttings were wrapped by hyphae, cuttings were healthy with green leaves with an increased height of approximately 4 cm (Fig. 4A), and with special hyphae structures on the wrapped roots (Fig. 4B–C). Stereomicroscope observations showed that hyphae was at the mulberry stage of fruiting body development, as there were a large number of white and brown particles called fruiting body primordium shaped like mulberries (Fig. 4D). Stereomicroscope observations also showed the hyphae were at the coral period of development where parts of the primordium continued to grow while other parts were shrinking. The growth primordium, that was thick on top and thin below, developed into primitive stalks shaped like coral. Meanwhile, the growth primordium shaped like a fan appearing at the top is called the primitive cap (Fig. 4E–F).



**Fig. 1:** Morphological characteristics of the fungus G18. **A:** Colony of G18 cultured on PDA medium for 7 days. **B:** Hyphae morphology and branching. **C:** Hyphae present at clamp connections (arrows). **D:** Hyphae tips with treelike branches. Scale bars: 1 cm (A), 100 μm (B), 20 μm (C, D)

```

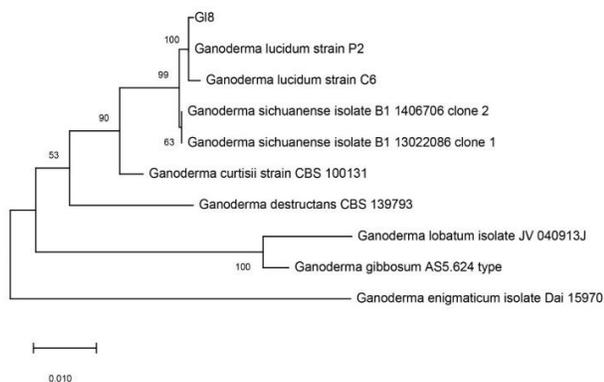
1  AGCCGTCGCTTGACGGGTGAGCTGGCCCTCCGAGGCATGTGCACGCCCTGCTCATCCAC
61  TCTACACCTGTGCACTTACTGTGGGCTTCAGATTGCGAGGCACGCTCTTTACCGGGCTTG
121  CGGAGCATATCTGTGCTGCGTTTATCACAACTCTATAAAGTAACAGAATGTGTAITGC
181  GATGTAACACATCTATATACAACTTTCAGCAACGGATCTCTGGCTCTCGCATCGATGAA
241  GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
301  TGAACGCACCTTGGCGCTCTTGGTATCCGAGGAGCATGCTGTTTGAGTGTGCATGAAAT
361  CTTCAACCTACAAGCTTTTGTGGTTTGTAGGCTTGGACTTGGAGGCTTGTGCGGCCGTAT
421  CGGTGGCTCCTCTAAATGCAATTAGCTTGGTTCCTTGCAGATCGGCTCTCGGTGTGATA
481  ACGTCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTCTAACCGCTCTATAAGACAGCTT
541  TATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACCTAAGCATATCAAAAAG

```

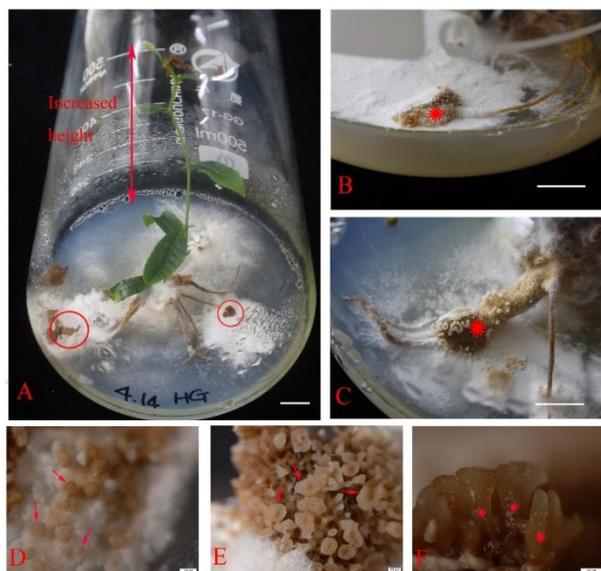
**Fig. 2:** ITS rDNA sequence of G18

### Anatomical Structure of Inoculated Roots

No hyphae were visible in the free-living root cuttings (Fig. 5A). Ten days after inoculation, microscopic observations of inoculated *C. henryi* cuttings showed that roots were infected by hyphae with intracellular hyphae growth observed in epidermal and cortical cells (Fig. 5B). TEM observations also showed that the hyphae inoculated the epidermal and cortical cells which maintained an intact cell wall, cytoplasm, and organelles, and no cellular disintegration (Fig. 5C and 5E); while the root hair cells were becoming deformed (Fig. 5D). Hyphae were observed to cross the cell membrane into another cell for proliferation, while no hyphae were observed in the apoplastic spaces, and the host cell walls were intact (Fig. 5E). Thirty days after inoculation, TEM showed that the hyphae colonized the intra- and intercellular spaces, and that root cells have obvious cytolysis (Fig. 5F).



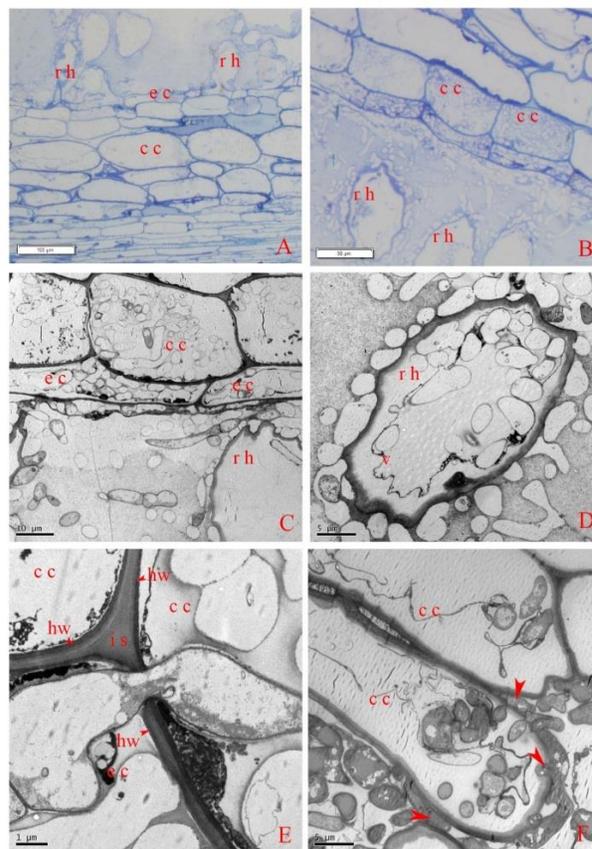
**Fig. 3:** Phylogenetic tree based on ITS rDNA sequence data of G18. The phylogenetic tree was constructed using the neighbor-joining method within MEGA software (version X). Numerical values above the branches indicate bootstrap percentiles from 1000 replicates, bootstrap numbers over 50% are indicated



**Fig. 4:** Photographs illustrating the fungus G18 development process when co-cultured with *C. henry* cuttings. **A:** Rooted cuttings were transferred to conical flasks, and their roots were inoculated by G18 mycelium disks. 10 days later, the seedlings have grown taller (increased height) and with special mycelium structure wrapped around the roots (circle). Scale bar: 1 cm. **B** and **C:** Closeup of the special mycelium structure (stars) shown in Figure 4A. Scale bars: 1 cm. **D:** Additional enlargement of the region circled in Figure 4A showing the fruiting body primordium shaped like mulberries (arrows). Scale bar: 200  $\mu$ m. **E:** Additional enlargement of the region circled in Figure 4A showing the primitive cap (arrows). Scale bar: 200  $\mu$ m. **F:** A medium longitudinal section of the region shown in Figure 4E showing the primitive stalk shaped like coral (stars). Scale bar: 200  $\mu$ m

## Discussion

The isolated strain reproduced using clamp connections, which were a hyphal protrusion that develop during cell



**Fig. 5:** Anatomical structures of *C. henry* root cuttings inoculated with G18. **A:** Control roots with no hyphae inoculation. Scale bar: 100  $\mu$ m. **B:** 10 days after inoculation, microscopic observation of hyphae-inoculated epidermal cells (ec) and cortical cells (cc). Scale bar: 50  $\mu$ m. **C:** TEM of hyphae-inoculated epidermal cells (ec) and cortical cells (cc). Scale bar: 10  $\mu$ m. **D:** Hyphae-inoculated root hair cells (rh). Scale bar: 5  $\mu$ m. **E:** An enlargement of the section shown in C. Hyphae crosses the cell membrane into another cell; hyphae branched inactively; only one septum in the whole area visible and no hyphae visible in the intercellular space (is). Host wall (hw; arrows) is intact. Scale bar: 1  $\mu$ m. **F:** As the infection progresses, there is widespread attack of the host cell walls (arrows) from intracellular and transmural hyphae. The hyphae colonize intra- and intercellular, root cells have cytoclasis

division to maintain the dikaryon condition (Krings *et al.*, 2011; Taylor *et al.*, 2014; Wan *et al.*, 2017). ITS rDNA analysis showed a high degree of sequence identity (99%) to *G. lucidum* strain P2 and C6 reported by Chen *et al.* (2017) which places the strain in one cluster in the neighbor joining phylogenetic tree. Thus, the strain was named *G. lucidum* G18.

*Ganoderma* species growth consists of several different stages, such as mycelium, primordium, and mature fruiting body (Zhou, 2017). *C. henry* cuttings promoted the G18 mycelium differentiation into the primordial stage of fruiting body development compared to the free-living *C. henry* cuttings. Thus, *C. henry* cuttings could supply some

necessary substance for G18 mycelium differentiation. However, further studies are needed to determine which substances from *C. henryi* contributed to mycelium differentiation.

The *in vitro* nutritional source for G18 development could be both *C. henryi* cuttings and the medium. On one hand, TEM indicated that the inoculated G18 epidermal and cortical cells had intact cell walls. Thus, it is very likely that the hyphae multiplied using nutrients from *C. henryi* and lived endophytically in *C. henryi*. This is supported by Abdullah (2000) who suggested that *G. boninense* is an endophyte in coconuts and by Panchal and Bridge (2005) who detected *Ganoderma* DNA in oil palms (*Elaeis guineensis* Jacq.) exhibiting no symptoms. On the other hand, TEM indicated that the root hair cells were becoming deformed. This phenomenon could be similar to the ectomycorrhizal fungi infecting the host roots causing root hair decay, and the fungi cells could replace root hair cells to absorb nutrients from the medium for plant and mycelium growth (Horan *et al.*, 1988; Ditengou *et al.*, 2000; Vayssières *et al.*, 2015).

Despite reports of some *Ganoderma* species causing wood decay and can be found on dead and living trees, there were many reports demonstrating that some *Ganoderma* species also act as a plant pathogen causing root and butt rot diseases on trees with high economic value such as oil palm, eucalyptus (*Eucalyptus pellita* Muell.), and ornamental forest trees (Paterson, 2007; Gill *et al.*, 2016; TchotetTchoumi *et al.*, 2018). Ten days after inoculation, TEM indicated that hyphae inoculated the epidermal and cortical cells with no cell disintegration and that host cell walls were still intact. Moreover, the inoculated cuttings had green leaves and an average height increase of approximately 4 cm indicating that G18 had not caused any disease. Therefore, G18 did not act as a plant pathogen at the early stages of *C. henryi* inoculation. Nevertheless, no fruiting bodies were observed in co-cultured or in free-living mycelium at the end of the study. Thirty days after inoculation, we observed that leaves were turning yellow, and TEM showed that hyphae colonized the intra- and intercellular spaces, and that root cells had obvious cytolysis, which seemed to indicate that G18 can act as a saprophyte or pathogen at this stage. Results in this study were similar to that by Rees *et al.* (2009) on the development and progress of basal stem rot in oil palm roots. Martin *et al.* (2015) found that 75% of the endophytic species in the wood of the genus *Hevea* were in the order Polyporales, which includes *Ganoderma* sp. Others reported that *G. zonatum* can live endophytically in palm trees; however, when tissues become weakened and susceptible to decay, *G. zonatum* would act as a latent saprophyte or pathogen (Martin *et al.*, 2015; Song *et al.*, 2017).

No mature fruiting bodies were observed in co-cultured and free-living mycelium at the end of the trial. The most logical reason could be that the nutrients and volume

in the conical flask are limiting factors for *C. henryi* cuttings and G18 primordium growth. In addition, the environment including light, humidity, and temperature might not be suitable for G18 to form fruiting bodies. Zhou (2017) reported that each development stage of *G. lucidum* has a unique set of requirements. Tree species produce and release antimicrobial compounds, such as phenolic compounds, resins, and tannins to resist decay caused by fungi (Scheffer and Cowling, 1966; Deflorio *et al.*, 2008; Rees *et al.*, 2009). The biosynthesis of antimicrobial compounds might be impeded under limited nutrition conditions. In addition, the roots of *C. henryi* cuttings are surrounded by a tough mycelium, which released plant cell wall degrading enzymes such as cellulase, laccases, and manganese peroxidases that are involved in the degradation of host cell wall (Rees *et al.*, 2009).

There were no reports on taxa of *Ganoderma* causing *C. henryi* death in nature; however, G18 almost acted as a pathogen on *C. henryi* *in vitro* at the end of this study. Boddy (2000) demonstrated that competition for allocation of a substrate by wood degrading fungi can occur in several ways including: chemical antagonism, mycoparasitism, and biological incompatibility. All these strategies can result with the displacement of a “vulnerable” fungus, or the hindrance of normal physiological function of a given fungus (Boddy, 2000). In *C. henryi* forested land in nature, there are many types of soil microbes, including wood decaying fungi, pathogenic fungi, and mycorrhizal fungi. The order Fagales is likely to be the oldest angiosperm ectomycorrhizal (ECM) group, and > 80% of the Fagales genera are ECM (Larson-Johnson, 2015; Tedersoo and Brundrett, 2017). Furthermore, there were reports showing that many *Castanea* species are ECM plant, including *C. mollissima* (Wan *et al.*, 2016), *C. sativa* (Martins *et al.*, 1996; Acioli-Santos *et al.*, 2008) and *C. henryi* (Liu *et al.*, 2016). ECM enhance nutrient uptake and increase host resistance to plant pathogens (Harely and Smith, 1983; Marx, 1969; Pfabel *et al.*, 2012). Therefore, G18 should not act as a pathogen on *C. henryi* in nature. All the results indicated that further studies are need to better understand the relationship between *C. henryi* and G18, and the conditions required for cultivation of G18 under *C. henryi* forested land.

## Conclusion

The present study isolated a new *G. lucidum* strain from fruiting bodies, which grown under mixed forests with Fagaceae as dominant tree species in south China, and we named it G18. The G18 was able to colonize the living roots of *C. henryi*, and the colonization permitted the G18 to form fruiting body primordium, primitive stalk, and primitive cap. This means that *C. henryi* live trees could potentially be used as a host of *G. lucidum* to improve the cultivation methods, as a new practice of agroforestry.

## Acknowledgments

This study was supported by Key Research and Development Program of Hunan Province (Grant No. 2018NK2043), the Chinese National Science and Technology Pillar Program (Grant No. 2013BAD14B04), and Scientific Innovation Fund for Post-graduates of Hunan Province (Grant No. CX2018B437).

## References

- Abdullah, F., 2000. Spatial and sequential mapping of the incidence of basal stem rot of oil palms (*Elaeis guineensis*) on a former coconut (*Cocos nucifera*) plantation. In: *Ganoderma Diseases of Perennial Crops*, pp: 183–194. Flood, J., P.D. Bridge and M. Holderness (Eds.). Wallingford, UK: CABI Publishing
- Acioli-Santos, B., M. Sebastiana, F. Pessoa, L. Sousa, A. Figueiredo, A.M. Fortes, A. Baldé, L.C. Maia and M.S. Pais, 2008. Fungal transcription pattern during the preinfection stage (12 h) of ectomycorrhiza formed between *Pisolithus tinctorius* and *Castanea sativa* roots, identified using cDNA microarrays. *Curr. Microbiol.*, 57: 620–625
- Boddy, L., 2000. Interspecific combative interactions between wood-decaying basidiomycetes. *FEMS Microbiol. Ecol.*, 31: 185–194
- Chen, B.Z., B.R. Ke, L.Y. Ye, S.S. Jin, F. Jie, L.L. Zhao and X.P. Wu, 2017. Isolation and varietal characterization of *Ganoderma resinaceum* from areas of *Ganoderma lucidum* production in China. *Sci. Hortic.*, 224: 109–114
- Čilerdžić, J., M. Stajić and J. Vukojević, 2016. Degradation of wheat straw and oak sawdust by *Ganoderma applanatum*. *Intl. Biodeter. Biodegr.*, 114: 39–44
- Deflorio, G., C. Johnson, S. Fink and F.W.M.R. Schwarze, 2008. Decay development in living sapwood of coniferous and deciduous trees inoculated with six wood decay fungi. *For. Ecol. Manage.*, 255: 2373–2383
- Dias, A.A., G.S. Freitas, G.S.M. Marques, A. Sampaio, I.S. Fraga, M.A.M. Rodrigues, D.V. Evtugin and R.M.F. Bezerra, 2010. Enzymatic saccharification of biologically pre-treated wheat straw with white-rot fungi. *Bioresour. Technol.*, 101: 6045–6050
- Ditengou, F.A., T. Béguiristain and F. Lapeyrie, 2000. Root hair elongation is inhibited by hypaphorine, the indole alkaloid from the ectomycorrhizal fungus *Pisolithus tinctorius*, and restored by indole-3-acetic acid. *Planta*, 211: 722–728
- Fan, X.M., D.Y. Yuan, X.M. Tian, Z.J. Zhu, M.L. Liu and H.P. Cao, 2017. Comprehensive transcriptome analysis of phytohormone biosynthesis and signaling genes in the flowers of Chinese chinquapin (*Castanea henryi*). *J. Agric. Food Chem.*, 65: 10332–10349
- Fan, X.M., D.Y. Yuan, J. Tang, X.M. Tian, L. Zhang, F. Zou and X.F. Tan, 2015. Sporogenesis and gametogenesis in Chinese chinquapin (*Castanea henryi* (Skam) Rehder and Wilson) and their systematic implications. *Trees*, 29: 1713–1723
- Gao, C., R. Yang and D. Yuan, 2018. Structural characteristics of the mature embryo sac of *Camellia oleifera*. *Nord. J. Bot.*, e01673
- Gill, W., A. Eyles, M. Glen and C. Mohammed, 2016. Structural host responses of *Acacia mangium* and *Eucalyptus pellita* to artificial infection with the root rot pathogen, *Ganoderma philippii*. *For. Pathol.*, 46: 369–375
- Hajjaj, H., C. Mace, M. Roberts, P. Niederberger and L.B. Fay, 2005. Effect of 26-oxygenosterols from *Ganoderma lucidum* and their activity as cholesterol synthesis inhibitors. *Appl. Environ. Microbiol.*, 71: 3653–3658
- Harely, J.L. and S.E. Smith, 1983. *Mycorrhizal Symbiosis*. Academic Press, Toronto
- Horan, D.P., G.A. Chilvers and F.F. Lapeyrie, 1988. Time sequence of the infection process in eucalypt ectomycorrhizas. *New Phytol.*, 109: 451–458
- Krings, M., N. Dotzler, J. Galtier and T.N. Taylor, 2011. Oldest fossil basidiomycete clamp connections. *Mycoscience*, 52: 18–23
- Kues, U., D.R. Nelson, C. Liu, G.J. Yu, J. Zhang, J. Li, X.C. Wang and H. Sun, 2015. Genome analysis of medicinal *Ganoderma* spp. with plant-pathogenic and saprotrophic life-styles. *Phytochemistry*, 114: 18–37
- Larson-Johnson, K., 2015. Phylogenetic investigation of the complex evolutionary history of dispersal mode and diversification rates across living and fossil Fagales. *New Phytol.*, 209: 418–435
- Liu, D.M., D.Y. Yuan, F. Zou, X.H. Zhang, Z.J. Zhu and L.M. Tan, 2016. Optimaization of culture condition for 3 *Castanea henryi* ectomycorrhizal fungi. *J. Northwest For. Univ.*, 31: 195–200
- Liu, D., J. Gong, W. Dai, X. Kang, Z. Huang and H.M. Zhang, 2012. The genome of *Ganoderma lucidum* provides insights into triterpenes biosynthesis and wood degradation. *PLoS One*, 7: e36146
- Loyd, A.L., B.W. Held, E.R. Linder, J.A. Smith and R.A. Blanchette, 2018a. Elucidating wood decomposition by four species of *Ganoderma* from the United States. *Fung. Biol.*, 122: 254–263
- Loyd, A.L., C.W. Barnes, B.W. Held, M.J. Schink, M.E. Smith, J.A. Smith and R.A. Blanchette, 2018b. Elucidating "lucidum": distinguishing the diverse laccate *Ganoderma* species of the United States. *PLoS One*, 13: e0199738
- Martin, R., R. Gazis, D. Skaltsas, P. Chaverri and D. Hibbett, 2015. Unexpected diversity of basidiomycetous endophytes in sapwood and leaves of *Hevea*. *Mycologia*, 107: 284–297
- Martins, A., J. Barroso and M.S. Pais, 1996. Effect of ectomycorrhizal fungi on survival and growth of micropropagated plants and seedlings of *Castanea sativa* mill. *Mycorrhiza*, 6: 265–270
- Marx, D.H., 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology*, 59: 153–163
- Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plantarum*, 15: 473–497
- Panchal, G. and P.D. Bridge, 2005. Following basal stem rot in young oil palm plantings. *Mycopathologia*, 159: 123–127
- Paterson, R.R.M., 2007. *Ganoderma* disease of oil palm—a white rot perspective necessary for integrated control. *Crop Prot.*, 26: 1369–1376
- Pfabel, C., K.U. Eckhardt, C. Baum, C. Struck, P. Frey and M. Weih, 2012. Impact of ectomycorrhizal colonization and rust infection on the secondary metabolism of poplar (*Populus trichocarpa* × *deltoides*). *Tree Physiol.*, 32: 1357–1364
- Rees, R.W., J. Flood, Y. Hasan, U. Potter and R.M. Cooper, 2009. Basal stemrot of oil palm (*Elaeis guineensis*); mode of root infection and lower stem invasion by *Ganoderma boninense*. *Plant Pathol.*, 58, 982–989
- Sanodiya, B., B.S. Sanodiya, G.S. Thakur, R.K. Baghel, G.B. Prasad and P.S. Bisen, 2009. *Ganoderma lucidum*: a potent pharmacological macrofungus. *Curr. Pharm. Biotechnol.*, 10: 717–742
- Santiago-Freijanes, J.J., A. Pisanelli, M. Rois-Díaz, J.A. Aldrey-Vázquez, A. Rigueiro-Rodríguez, A. Pantera, A. Vityi, B. Lojka, N. Ferreira-Domínguez and M.R. Mosquera-Losada, 2018. Agroforestry development in Europe: Policy issues. *Land Use Policy*, 76: 144–156
- Scheffer, T.C. and E.B. Cowling, 1966. Natural resistance of wood to microbial deterioration. *Annu. Rev. Phytopathol.*, 4: 147–168
- Shi, L., A. Ren, D. Mu and M.W. Zhao, 2010. Current progress in the study on biosynthesis and regulation of ganoderic acids. *Appl. Microbiol. Biotechnol.*, 88: 1243–1251
- Shiao, M.S., 2003. Natural products of the medicinal fungus *Ganoderma lucidum*: occurrence, biological activities, and pharmacological functions. *Chem. Rec.*, 3: 172–180
- Song, Z., P.G. Kennedy, F.J. Liew and J.S. Schilling, 2017. Fungal endophytes as priority colonizers initiating wood decomposition. *Funct. Ecol.*, 31: 407–418
- Taylor, H.C., 1969. Pest plants and nature conservation in the winter rainfall region. *J. Bot. Soc. S. Afr.*, 55: 32–35
- Taylor, T.N., M. Krings and E.L. Taylor, 2014. *Fossil Fungi*, 1<sup>st</sup> edition, p: 398. Elsevier/Academic Press Inc., San Diego California, USA

- Tchoumi, J.M.T., M.P.A. Coetzee, M. Rajchenberg, M.J. Wingfield and J. Roux, 2018. Three *Ganoderma* species, including *Ganoderma dunense* spp. nov., associated with dying *Acacia cyclops* trees in South Africa. *Aust. Plant Pathol.*, 47: 431–447
- Tedersoo, L. and M.C. Brundrett, 2017. Evolution of ectomycorrhizal symbiosis in plants. In: *Biogeography of Mycorrhizal Symbiosis Ecological Studies*, pp: 407–467. Tedersoo, L. (Ed.). Springer International Publishing, Dordrecht, Netherlands
- Vayssières, A., A. Pěnčík, J. Felten, A. Kohler, K. Ljung, F. Martin and V. Legué, 2015. Development of the Poplar-*Laccaria bicolor* ectomycorrhiza modifies root auxin metabolism, signaling, and response. *Physiol. Plantarum*, 169: 890–902
- Wan, M., W. Yang, X. He, L. Liu and J. Wang, 2017. First record of fossil basidiomycete clamp connections in cordaitalean stems from the Asselian-Sakmarian (lower Permian) of Shanxi Province, North China. *Palaeoogeogr. Palaeoclimatol. Palaeoec.*, 466: 353–360
- Wan, S.P., F.Q. Yu, L. Tang, R. Wang, Y. Wang, P.G. Liu, X.H. Wang and Y. Zheng, 2016. Ectomycorrhizae of *Tuber huidongense* and *T. liyuanum* with *Castanea mollissima* and *Pinus armandii*. *Mycorrhiza*, 26: 249–256
- Wood, A.R. and J. Ginns, 2006. A new dieback disease of *Acacia cyclops* in South Africa caused by *Pseudolagarobasidium acaciicola* spp. nov. *Can. J. Bot.*, 84: 750–758
- Wu, Y.Q., X.R. Mao, L.W. Yao, W.L. Chen, Z.W. Xue and G.H. Ying, 2018. Experiment on different formula of substrate and cultivation measures on yield of *Dictyophora echinovolvata* under *Castanea henryi* Stand. *J. Zhej. For. Sci. Technol.*, 38: 59–62
- Xing, J.H., Y.F. Sun, Y.L. Han, B.K. Cui and Y.C. Dai, 2018. Morphological and molecular identification of two new *Ganoderma* species on *Casuarina equisetifolia* from China. *Myckeys*, 34: 93–108
- Xiong, H., Z.Q. Liu, F. Zou and D.Y. Yuan, 2018a. Seasonal variation of nutrient content of leaves and soil in *Castanea henryi*. *Southwest Chin. J. Agric. Sci.*, 31: 1405–1410
- Xiong, H., H. Sun, F. Zou, X.M. Fan, G.H. Niu and D.Y. Yuan, 2018b. Micropropagation of Chinquapin (*Castanea henryi*) Using Axillary Shoots and Cotyledonary Nodes. *HortScience*, 53: 1482–1486
- Yang, Z., J. Feng and H. Chen, 2013. Establishment of tissue culture regeneration system for *Castanea henryi*. *J. Fruit Sci.*, 30: 105–109
- Yao, L.W., M.L. Lv, Y.Q. Wu, X.R. Mao, W.L. Chen and Z.W. Xue, 2018. Study on different compound planting *Dictyophora indusiatao* and *Polygonatum cyrtonea* under *Castanea henryi* (Skan) Rehd. et Wils. Stand. *South Chin. For. Sci.*, 46: 57–59
- Zhang, G., A. Ren, L. Shi, J. Zhu, A.L. Jiang, D.K. Shi and M.W. Zhao, 2018. Functional analysis of an APSES transcription factor (*GISwi6*) involved in fungal growth, fruiting body development and ganoderic-acid biosynthesis in *Ganoderma lucidum*. *Microbiol. Res.*, 207: 280–288
- Zhang, G., Z.H. Sun, A. Ren, L. Shi, D.K. Shi, X.B. Li and M.W. Zhao, 2017. The mitogen-activated protein kinase *GISit2* regulates fungal growth, fruiting body development, cell wall integrity, oxidative stress and ganoderic acid biosynthesis in *Ganoderma lucidum*. *Fung. Genet. Biol.*, 104: 6–15
- Zhang, W.Q. and Q.Z. Yao, 2019. Morpho-anatomical characterization and phylogenetic analysis of five *Tomentella* ectomycorrhizae from leigong mountain, Guizhou. *Intl. J. Agric. Biol.*, 21: 853–858
- Zhang, W.R., S.R. Liu, Z.X. Zhao, Y.B. Kuang, X.F. Dong and J.F. Ruan, 2018. Effects of extracts of spent mushroom substrates on growth of edible fungi. *Intl. J. Agric. Biol.*, 20: 2133–2139
- Zheng, C., X. Zhang, N. Huang, Y. Jiang, 2002. Preliminary study on analyses of nutrient ingredients in nuts of different chinquapin (*Castanea henryi*) cultivars. *Subtrop. Plant Sci.*, 31: 5–8
- Zhou, X.W., 2017. Cultivation of *Ganoderma lucidum*. In: *Edible and Medicinal Mushrooms: Technology and Applications*, First Edition, pp: 385–413. Zied, D. C. and A. Pardo-Giménez (Eds.). John Wiley & Sons Ltd, New York, USA

[Received 02 Feb 2019; Accepted 12 Mar 2019; Published (online) 12 Jul 2019]