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ARTICLE

Medicinal Mushroom *Ganoderma lucidum* as a Potent Elicitor in Production of *t*-Resveratrol and *t*-Piceatannol in Peanut Calluses

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Phytoalexins *t*-resveratrol and *t*-piceatannol, the well-known health-promoting active components in plants, are secondary metabolites generated upon biotic or abiotic stresses. We have reported UV-irradiated peanut callus is a potent means to produce these compounds (*J. Agric. Food Chem.* **2005**, *53*, 3877). In this work, the effects of fungi and chemical elicitors on induction of *t*-resveratrol and *t*-piceatannol were examined. Results showed the investigated fungi *Botryodiplodia theobromae* and Reishi *Ganoderma lucidum* were generally more effective than chemical stress methyl jasmonate, salicylic acid, and sucrose. As high as 15.46 ± 9.85 μg of *t*-resveratrol and 6.93 ± 2.03 μg of *t*-piceatannol could be elicited in each gram of callus by sterilized *G. lucidum* mycelium (80 mg). Although much more sterilized *G. ludicum* mycelia was required to induce similar level of *t*-resveratrol and *t*-piceatannol in comparison to the sterilized *B. theobromae* mycelia (1 mg), uptake of the *G. ludicum* mycelium may provide a variety of health-promoting effects. Our findings suggest *G. ludicum* mycelium-treated peanut callus is a good source of bioactive components.

KEYWORDS: Fungi; Reishi *G. lucidum*; *t*-resveratrol; *t*-piceatannol; peanut callus

INTRODUCTION

Plants, against biotic or abiotic stress, usually synthesize phytoalexins through various signal transduction paths (*2*). Some of these secondary metabolites draw attention from pharmaceutical and food industry because of great biological activities. For instance, *t*-resveratrol and *t*-piceatannol have been extensively studied and well-known of health-promoting functions such as anti-inflammatory, anticarcinogenic, antiviral, antioxidative, neuroprotective, and estrogenic properties (*3*−*9*).

Grape and peanut are the most common sources of *t*-resveratrol and *t*-piceatannol in our daily diet in spite of the great variation with species and processing. The maximal contents of *t*-resveratrol and *t*-piceatannol in seven varieties of grapes were reported 666 and 41 μg/100 g berries, respectively (*10*). Each liter of red wine contains 0.99−5.01 mg of *t*-resveratrol (*11*). The concentration of *t*-resveratrol in raw peanut kernels, roast peanut, and boiled peanut found was 0.48 ± 0.08 μg/g dry weight (*12*), 0.055 ± 0.023 μg/g, and 5.138 ± 2.849 μg/g, respectively (*13*).

A great deal of effort has been made to enhance the production of stilbenoids. With abiotic stresses such as UV irradiation, ultrasound exposure, and gas treatment, the best yield of *t*-resveratrol and *t*-piceatannol obtained in grape were 2315.9 ± 190.4 and 173.4 ± 15.6 μg/100 g of berries, respectively (*10*, *14*−*16*). Meanwhile, the production of *t*-resveratrol and *t*-piceatannol in peanut was increaced to 147.3 ± 14.0 μg/g of dry seeds and 5.31 ± 0.51 μg/g of fresh calluses, respectively (*1*, *12*, *17*).

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Nevertheless, the more successful induction of *t*-resveratrol was achieved by artificial microbial infection. Paul et al. (*18*) found simultaneous inoculation of *Botrytis cinerea* spores and soil bacteria could increase the content of *t*-resveratrol to 31.06 μg/g of fresh grape leaves. The production of *t*-resveratrol in peanut kernels was raised to mg/g level in response to aflatoxin-producing *Aspergillus* infection (*19*). We also found that fungi elicited the production of *t*-resveratrol and *t*-piceatannol in peanut callus more efficiently than Gram (−) bacteria (*20*). However, all the fungi reported as elicitors are plant pathogens and might be unsuitable for food use. In this regard, this work was aimed to develop an alternative approach by using edible fungi Reishi, which is generally recognized as safe (GRAS), as the elicitor. The inductive effects of different fungi, a peanut pathogen *Botryodiplodia theobromae* LBBT HC6-1 and the health-promoting Reishi *Ganoderma lucidum*, were compared. Three commonly used abiotic stressors methyl jasmonate, salicylic acid, and sucrose were also applied to peanut calluses for induction of *t*-resveratrol and *t*-piceatannol.

MATERIALS AND METHODS

1. Reagents. *t*-Resveratrol, *t*-piceatannol, methanol, acetonitrile, and formic acid were purchased from Merck (Darmstadt, Germany). Methyl jasmonate and salicylic acid were obtained from Sigma-Aldrich (Missouri). Sucrose was from Taisuco, Taiwan.

2. Callus Culture. Peanut calluses were obtained from the seeds of *Arachis hypogaea* L. cv. Tainan no. 9 (Tainan District Agricultural Research and Extension Station, Taiwan) and prepared according to the procedure described by Ku et al. (*1*) with modification. Briefly, deshelled

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Table 1. Dose Effects of *Botryodiplodia theobromae* Spore Suspension on the Elicitation of *t*-Resveratrol and *t*-Piceatannol in Peanut Calluses after Incubation at 28 °C for 24 h as Described in Materials and Methods[a]

	t-resveratrol (μg/g fresh calluses)		*t*-piceatannol (μg/g fresh calluses)	
dose (conidia)	viable spores	sterilized spores	viable spores	sterilized spores
0	ND	ND	ND	ND
10^3	4.52 ± 2.25 ab	5.46 ± 2.68 b	2.03 ± 0.57 b	0.47 ± 0.14 d
10^4	5.02 ± 0.85 ab	7.75 ± 2.76 b	6.67 ± 2.99 a	2.20 ± 0.71 bc
10^5	7.08 ± 3.51 a	19.14 ± 4.36 a	9.61 ± 3.21 a	4.10 ± 1.15 a
10^6	5.81 ± 3.29 a	12.27 ± 2.17 ab	1.28 ± 0.56 b	3.36 ± 0.57 ab
10^7	1.03 ± 0.34 b	4.74 ± 1.13 b	0.17 ± 0.08 b	1.10 ± 0.56 cd

[a] Data were shown as mean ± SD (n = 3). ND, not detectable. Values followed by different letters in the same column were significantly different as determined by LSD test at $P \leq 0.05$.

seeds were rinsed with deionized water, disinfected by sonication in 100 mL of 1% sodium perchlorite solution containing 1 drop of Tween 20 for 1 min, and washed with sterile deionized water. The cleaned seeds were grown in Murashige and Skoog's basal medium for 1 week. Then the hypocotyls was aseptically cut into explants of 1 cm size and cultured on agar containing MS medium, 30 g/L of sucrose, 0.25 mg/L of 1-naphthylacetic acid, and 0.075 mg/L of 6-benzylaminopurine (pH 5.8) at 28 °C in the dark for 1 week or so before subjected to elicitation.

3. Treatment of Calluses. *3.1. Biotic Elicitors. Botryodiplodia theobromae* LBBT HC6-1 was isolated from lima bean and maintained in our laboratory. *Ganoderma lucidum* (Curtis: Fries) Karsten BCRC35398 was obtained from a mushroom farm in Jhongpu, Chiayi. Original mycelia were aseptically cut from basidiocarps of *G. lucidum* and cultured on potato dextrose agar (PDA) medium. Masses of mycelia were cut by a puncher (5 mm diameter) and subcultured on PDA at 25 °C for 3 days (*B. theobromae*) or 8 days (*G. lucidum*). To obtain mycelium suspensions, mycelia were scraped off, homogenized in sterile water, and adjusted to 0.05−40 mg/mL (*B. theobromae*) or 0.25−80 mg/mL (*G. lucidum*). One mL of each mycelium suspension was applied onto peanut calluses (ca. 1 g) and incubated at 28 °C in the dark for 24 h. Sterilized mycelium suspensions were similarly prepared except the scraped mycelia were autoclaved (121 °C, 15 lb/in^2, 15 min) prior to homogenization. *B. theobromae* spores were washed off with sterile water after incubation on potato agar for 10 days and adjusted to 10^3-10^7 conidia/mL. Spores were boiled for 15 min for sterilization. One mL of viable and sterilized spore suspension at each level was separately applied to 1 g of peanut callus for induction of stilbenoids at 28 °C in the dark. In the time-course study, calluses under treatment were sampled every 6 h up to 54 h of incubation. Sterile water (1 mL) was used as the control.

3.2. Abiotic Elicitors

3.2.1. Methyl Jasmonate (MeJA) and Salicylic Acid (SA). MeJA and SA were dissolved in dimethyl sulfoxide (Hayashi Pure Chemical, Osaka, Japan) and ethyl alcohol, respectively. After filtration through a 0.22 μm filter, each stock solution was diluted to 1, 5, 10, 15, and 25 μg/mL with the same solvent. One mL of DMSO (the control for MeJA), ethyl alcohol (the control for SA), and each diluted solution was separately applied onto peanut calluses (ca. 1 g) and incubated at 28 °C in the dark for 24 h.

3.2.2. Sucrose. Sucrose was dissolved in sterile double-distilled water and diluted to 30, 60, 90, 120, and 150 mg/mL. One mL of sterile water and the sucrose solution at each concentration was separately sprayed onto peanut calluses (ca. 1 g) and incubated at 28 °C in the dark for 4 weeks.

4. Extraction of Stilbenoids and HPLC Analysis. About one gram of callus was repetitively ground in 1 mL of methanol for three times to extract stilbenoids. The filtrate was combined and adjusted to 5 mL.

Quantitative determination of *t*-resveratrol and *t*-piceatannol in methanol extracts was performed by RP-HPLC with a fluorescence detector (λ_{ex} = 343 nm, λ_{em} = 395 nm) as previously described by Ku et al. (*1*).

5. Statistical Analysis. Data were analyzed by analysis of variance using least significant difference test. Differences were considered significant at $P \leq 0.05$ (SAS program Windows version 6.2). All values were presented as mean ± SD.

RESULTS AND DISCUSSION

1. Effects of *Botryodiplodia theobromae* LBBT HC6-1 Spores on the Induction of Stilbenoids in Peanut Calluses. *t*-Resveratrol and *t*-piceatannol were successfully induced in peanut calluses incubated with *Botryodiplodia theobromae* spores at five different levels (**Table 1**). In the presence of viable spores, the contents of *t*-resveratrol and *t*-piceatannol were increased to 1.03−7.08 and 0.17−9.61 μg per gram of fresh calluses, respectively. Even more *t*-resveratrol (4.74−19.14 μg/g) was elicited by sterilized spores at the same level, whereas less *t*-piceatannol (0.47−4.10 μg/g) was induced by 10^3-10^5 sterilized spores. Regardless of viability, there was a common trend that elicitation of stilbenoids reached the maximum when the dose was increased to 10^5 conidia. With higher amount of viable spores, the growth of calluses was obviously hindered and the calluses were tangled by mycelia to soon become withered. The fact that overloads of fungal elicitors inversed the production of stilbenoids and even caused callus death is noticeable. Because the phenomenon was commonly observed regardless of fungal viability or sterilization, pathogenic infection should be excluded. On the basis of our results, chitin, the key component of fungal cell wall, was suspected as the causal factor because chitin can act as the signaling factor in plant disease resistance. It is well-known that upon fungal challenge plants usually synthesize chitinases to destroy the invasive pathogens and to produce chitin fragments (chitin oligosaccharides), which in turn, initiating signaling pathways (*21*). A chitin oligosaccharide elicitor-binding protein (CEBiP) was isolated from the plasma membrane of suspension-cultured rice cells and found relevant to the biosynthesis of phytoalexins and formation of reactive oxygen species (ROS). Knockdown of CEBiP gene resulted in the decrease of ROS along with the cancellation of up-regulation of genes encoding enzymes such as phenylalanine ammonia lyase (PAL) and caffeoyl-CoA 3-*O*-methyltransferase (*22*, *23*). It is suggested that overloads of fungal chitin in peanut callus culture greatly induced ROS generation, leading to hypertensive response and even programmed cell death as seen in other leguminous plants (*24*).

Viable spores of another fungus *Aspergillus caelatus* were utilized to infect viable peanut seeds on the ratio of 10^7 spores/6 g seeds, 16 times our most effective dose. After 24 h of incubation, 12 stilbenoids including *t*-resveratrol were identified and the overall *t*-stilbenoid production was estimated at 2.1 mg per g fresh seeds (*25*). Although it is hard to compare the elicitation effect of *A. caelatus* with our result because the amount of individual *t*-stilbenoid, especially *t*-resveratrol and *t*-piceatannol, was not reported, the order of appearance is similar to our results, i.e., *t*-resveratrol prior to *t*-piceatannol.

When peanut calluses were separately inoculated with viable and sterilized *Botryodiplodia theobromae* spores at the most effective dose, 10^5 conidia, the time course of the elicitation of *t*-resveratrol and *t*-piceatannol, was monitored. The maximal production of *t*-resveratrol was found at the 12 h point and *t*-piceatannol at the 24 h point, regardless of the viability of elicitors (**Figure 1**).

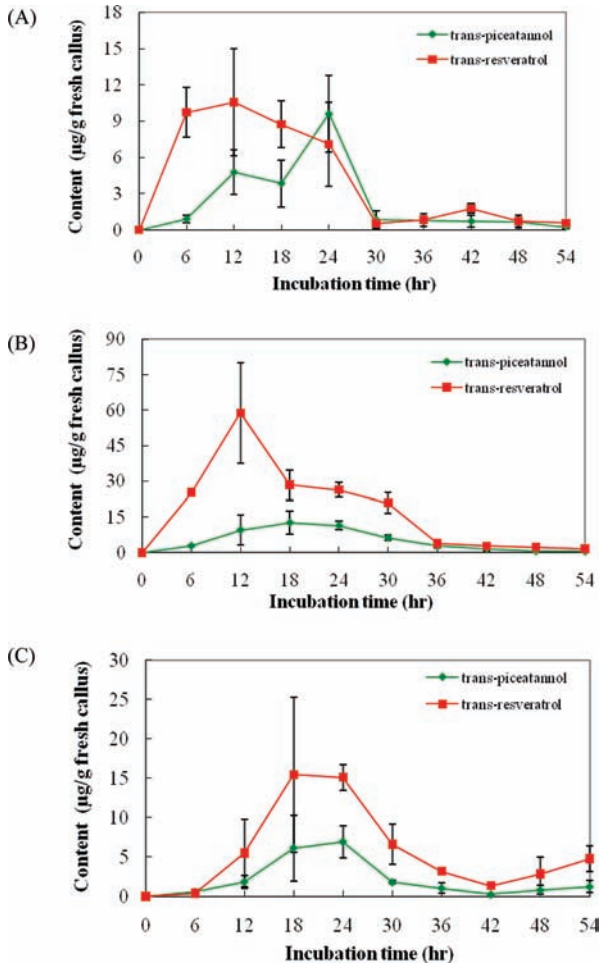

Figure 1. Time course of the elicitation of *t*-resveratrol and *t*-piceatannol in peanut calluses by 1 mL of (**A**) viable and (**B**) sterilized *B. theobromae* LBBT HC6-1 spore suspension (10^5 conidia/mL) (n = 6), (**C**) sterilized *G. lucidum* mycelium suspension (80 mg/mL) (n = 3).

Table 2. Dose Effects of *Botryodiplodia theobromae* Mycelium Suspension on the Elicitation of *t*-Resveratrol and *t*-Piceatannol in Peanut Calluses after 24 h Incubation As Described in Materials and Methods[a]

dose	*t*-resveratrol (μg/g fresh calluses)		*t*-piceatannol (μg/g fresh calluses)	
(mg)	viable mycelia	sterilized mycelia	viable mycelia	sterilized mycelia
0	ND	ND	ND	ND
0.05	4.42 ± 4.01 bc		0.56 ± 0.14 b	
0.15	6.47 ± 2.09 b		1.75 ± 0.54 b	
0.25	12.34 ± 7.48 a	3.79 ± 1.52 b	6.80 ± 3.86 a	1.56 ± 0.51 a
0.5	3.05 ± 0.89 bc	3.44 ± 0.63 b	1.27 ± 0.71 b	0.99 ± 0.18 a
1	3.06 ± 0.51 bc	12.88 ± 8.72 a	0.97 ± 0.44 b	2.30 ± 1.51 a
5	3.28 ± 1.45 bc	3.23 ± 0.94 b	0.63 ± 0.21 b	1.42 ± 0.74 a
10	1.62 ± 0.91 bc	5.14 ± 1.31 b	0.54 ± 0.34 b	1.53 ± 0.60 a
20	0.48 ± 0.40 c	4.77 ± 2.10 b	0.28 ± 0.24 b	1.30 ± 0.37 a
40	0.06 ± 0.03 c	4.71 ± 2.03 b	0.11 ± 0.08 b	0.95 ± 0.34 a

[a] Data were shown as mean ± SD (n = 3). ND, not detectable. Values followed by different letters in the same column were significantly different as determined by LSD test at $P \leq 0.05$.

Because *t*-piceatannol, 3,4,3′,5′-tetrahydroxy-*trans*-stilbene, has one more hydroxyl group on the 3′-position than *t*-resveratrol and the time course showed *t*-piceatannol was synthesized later than *t*-resveratrol, it is implicated that *t*-resveratrol is easily induced by contact of fungal spores, but the unknown key enzyme for *t*-piceatannol synthesis may need further stimulation such as invasion of the viable spore and mycelia. Sobolev (2008) have studied the distribution of induced phytoalexins in peanut kernels by infection of *Aspergillus* strains including the aflatoxin-generating *A. flavus* and *A. parasiticus* (*19*). He found that in the first 24 h of incubation, *t*-resveratrol was almost singularly found in tissues far away from the infected area, whereas five other more complicated phytoalexins *t*-arachidin-1, *t*-arachidin-2, *t*-arachidin-3, *t*-3-isopentadienyl-4, 3′, 5′-trihydroxystilbene, and SB-1 were found in areas close to the infected surface. With increasing incubation time, more phytoalexins in addition to *t*-resveratrol appeared in the remote area. These results also implied that *t*-resveratrol is synthesized prior to other stilbenoids.

Table 3. Dose Effects of *Ganoderma lucidum* Mycelium Suspension on the Elicitation of *t*-Resveratrol and *t*-Piceatannol in Peanut Calluses after 24 h Incubation As Described in Materials and Methods[a]

dose	*t*-resveratrol (μg/g fresh calluses)		*t*-piceatannol (μg/g fresh calluses)	
(mg)	viable mycelia	sterilized mycelia	viable mycelia	sterilized mycelia
0	ND	ND	ND	ND
0.25	2.31 ± 1.70 b	3.02 ± 1.41 b	0.81 ± 0.20 b	0.45 ± 0.12 d
0.5	3.69 ± 1.56 ab	3.70 ± 1.88 b	0.84 ± 0.61 b	0.72 ± 0.46 d
1	4.18 ± 1.97 ab	3.68 ± 1.35 b	0.68 ± 0.08 b	0.51 ± 0.37 d
5	4.30 ± 1.13 ab	3.12 ± 0.85 b	1.35 ± 0.81 b	0.73 ± 0.11 d
10	6.03 ± 2.11 a	5.52 ± 0.57 b	2.88 ± 2.08 a	1.46 ± 1.28 cd
20	4.14 ± 1.91 ab	5.53 ± 0.36 b	0.88 ± 0.33 b	2.00 ± 0.46 bc
40	5.16 ± 2.28 ab	11.37 ± 0.64 a	0.91 ± 0.53 b	2.81 ± 0.99 b
80	4.40 ± 0.62 ab	14.69 ± 3.01 a	0.76 ± 0.22 b	4.13 ± 1.09 a
120		5.09 ± 1.26 b		1.04 ± 0.24 cd

[a] Data were shown as mean ± SD (n = 3). ND, not detectable. Values followed by different letters in the same column were significantly different as determined by LSD test at $P \leq 0.05$.

2. Elicitation of Stilbenoids by Medicinal Mushroom *Ganoderma lucidum*. **Table 2** shows the induction of stilbenoids by *B. theobromae* mycelium was dose-dependent with viable mycelium more effective than the sterilized mycelium. The maximal *t*-resveratrol (12.34 ± 7.48 μg/g) and *t*-piceatannol (6.80 ± 3.86 μg/g) were induced by viable *B. theobromae* mycelia at the dose of 0.25 mg. After sterilization, more mycelia (1 mg) were needed for the most significant elicitation of *t*-resveratrol (12.88 ± 8.72 μg/g) and *t*-piceatannol (2.30 ± 1.51 μg/g).

Obviously, all the impressive results including those reported by the other teams indicated that stilbenoids could be dramatically elicited by fungi. However, great effort has to be made for extraction and purification in order to avoid dangerous constituents in the fungi-merged calluses before any further utilization. Considering potential application with less processing, we sought the generally recognized as safe (GRAS) medicinal mushroom Reishi *G. lucidum* as an elicitor.

When Reishi *G. lucidum* mycelia were used to examine the dose effect, production of stilbenoids was increased with the increasing addition of mycelia up to 10 mg of viable mycelia and 80 mg of sterilized mycelia, whereas adversely decreased with even more mycelia (**Table 3**). In contrast to the better effect of viable *B. theobromae* mycelia, the maximal *t*-resveratrol induced by viable *G. lucidum* mycelia (6.03 ± 2.11 μg/g) was about 40% of the amount induced by sterilized *G. lucidum* mycelia (14.69 ± 3.01 μg/g), and the maximal *t*-piceatannol induced by viable mycelia (2.88 ± 2.08 μg/g) was about 70% of those induced by sterilized mycelia (4.13 ± 1.09 μg/g). In the time-course study using 80 mg of sterilized *G. lucidum* mycelia as the elicitor, the maximal content of *t*-resveratrol (15.46 ± 9.85 μg/g) and *t*-piceatannol (6.93 ± 2.03 μg/g) was found at the 18 h and 24 h time point, respectively (**Figure 1C**). The color of calluses treated with sterilized *B. theobromae* mycelia changed from light yellow at 12 h (**Figure 2A**) to strong yellow at 24 h (**Figure 2B**) due to the accumulation of stilbenoids. In comparison with the slightly yellowish color of

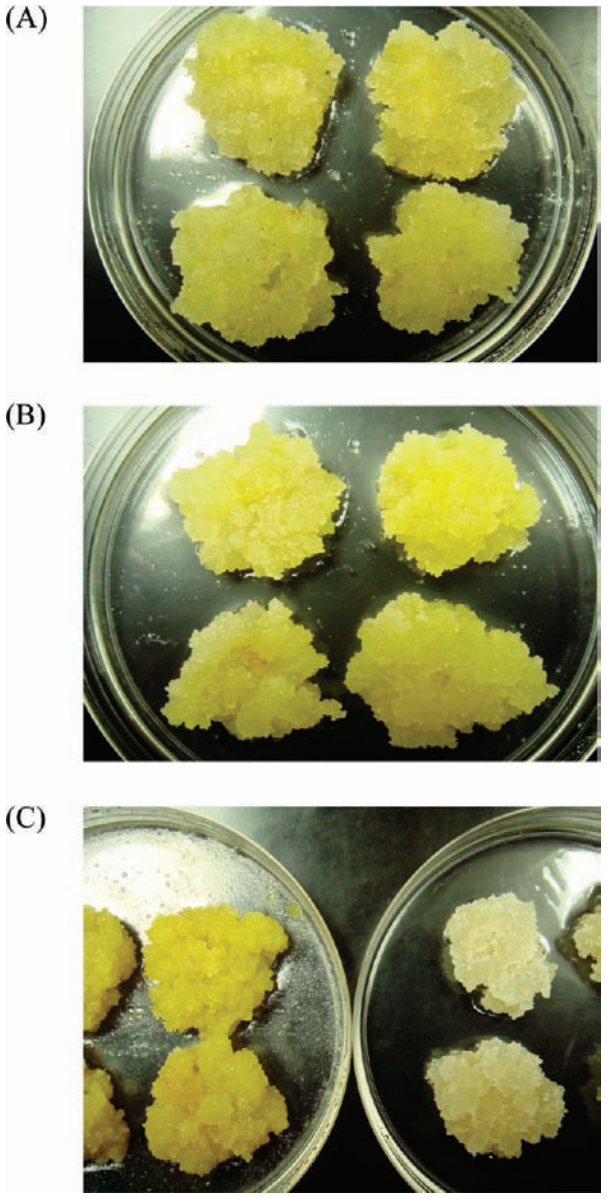

Figure 2. Appearance of peanut calluses after elicitation with 1 mL of sterilized *Botryodiplodia theobromae* LBBT HC6-1 spores (10^5 conidia/mL) for (**A**) 12 h, (**B**) 24 h, and (**C**) with 1 mL of sterilized *Ganoderma lucidum* mycelia (80 mg/mL) for 24 h, left; control, right.

the blank (**Figure 2C**, right), the appearance of the calluses treated with sterilized *G. lucidum* was significantly different (**Figure 2C**, left).

In spite of the fact that the most effective dose of sterilized *G. ludicum* mycelia (80 mg) was much more than that of the sterilized *B. theobromae* mycelia (1 mg), uptake of *G. lucidum* mycelium may additionally provide a variety of health-promoting effects (*26*). Water extract of *G. ludicum* mycelia was found hypotensive in rabbits and rats due to its central inhibition of sympathetic nerve activity (*27*). A triterpene-enriched fraction from the mycelia of *G. lucidum* was reported to inhibit the growth of human hepatoma cells, but no effect on the normal liver cells, via suppressing protein kinase C and activating mitogen-activated protein kinases and G2-phase cell cycle arrest (*28*). Furthermore, a proteoglycan isolated from the mycelia of *G. lucidum* grown on PDA medium was demonstrated in vitro as a potential agent against HSV-1 and HSV-2 (*29*, *30*) and later also found to be protective against carbon tetrachloride-induced liver injury in vitro and in vivo (*31*). Purified polysaccharides from *G. lucidum* mycelia could induce human peripheral blood mononuclear cell proliferation and maturation of dendritic cells with significant IL-12 and IL-10 production (*32*). Chien et al. (*33*) reported that both alcohol and water extracts of the liquid cultivated mycelia of *G. lucidum* inhibited tyrosinase activity, the key enzyme in melanin formation, with IC_{50} about 0.32 mg/mL. The cytotoxicity of the ethanol extract obtained from the fermentation mycelia of *G. lucidum* against Hep 3B carcinoma cells has been investigated (*34*). After fractionation of the crude extract, two active components, 9,11-dehydroergosterol peroxide (IC_{50} value 16.7 μg/mL) and ergosterol peroxide (IC_{50} value 19.4 μg/mL), were identified.

Table 4. Production of Stilbenoids in Peanut Calluses Induced by Fungi and Chemicals[a]

	maximal production (μg/g fresh calluses)	
elicitors (per gram of callus)	*t*-resveratrol	*t*-piceatannol
B. theobromae spores		
viable, 10^5 conidia	10.57 ± 4.46 (12 h)	9.66 ± 3.17 (24 h)
sterilized, 10^5 conidia	58.84 ± 21.16 (12 h)	18.69 ± 2.52 (24 h)
B. theobromae mycelia		
viable, 0.25 mg	14.43 ± 8.55 (12 h)	6.80 ± 3.86 (24 h)
sterilized, 1 mg	12.88 ± 8.72 (24 h)	2.30 ± 1.51 (24 h)
G. lucidum mycelia		
viable, 10 mg	6.03 ± 2.11 (24 h)	2.88 ± 2.08 (24 h)
sterilized, 80 mg	15.46 ± 9.85 (18 h)	6.93 ± 2.03 (24 h)
methyl jasmonate, 15 μg	2.55 ± 1.24 (24 h)	0.50 ± 0.09 (24 h)
salicylic acid, 20 μg	1.14 ± 0.42 (24 h)	0.35 ± 0.09 (24 h)
sucrose, 30 mg	0.15 ± 0.03 (1 wk)	5.40 ± 0.24 (1 wk)

[a] Data were shown as mean ± SD (n = 3). Values in parentheses indicated incubation time.

The dose effect of *G. lucidum* on stilbenoid elicitation in peanut calluses was similar to that on salidroside elicitation in *Rhodiola sachalinensis*. When *G. lucidum* was added to the root culture of *Rhodiola sachalinensis* at three levels (0.025, 0.05, and 0.1 mg/L), maximal elicitation was achieved at the medial level where the salidroside content was increased from 0.42% of dry weight in the control to 0.71% (*35*). Fortunately, the most effective dose of *G. lucidum* on stilbenoid elicitation can obtain a better inductive effect (1.4% fw).

3. Comparison of the Effects of Fungi and Chemicals. **Table 4** summarizes the maximal production of *t*-resveratrol and *t*-piceatannol induced by fungi and chemicals after a series of systematic investigation. In general, fungi are more effective than commonly used chemical elicitors methyl jasmonate and salicylic acid. Sucrose, the potent signaling compound in plants (*36*), was even less effective but surprisingly induced more *t*-piceatannol (5.40 ± 0.24 μg/g) than *t*-resveratrol (0.15 ± 0.03 μg/g) after incubation for 1 week. Although the maximal production of *t*-resveratrol (58.84 ± 21.16 μg/g) and *t*-piceatannol (18.69 ± 2.52 μg/g) was elicited by sterilized *B. theobromae* spores (10^5 conidia), elicitation by sterilized *G. lucidum* mycelium (80 mg) with the aforementioned biological activities may be more beneficial if mycelia are consumed with peanut calluses containing stilbenoids.

Meanwhile, the maximal quantity of *t*-resveratrol was usually found earlier than that of *t*-piceatannol (**Table 4**), implying *t*-piceatannol, 3,4,3′,5′-tetrahydroxy-*trans*-stilbene, was synthesized later than *t*-resveratrol with one less hydroxyl group on the 3′-position. Unfortunately, the enzyme(s) involved in *t*-piceatannol synthesis is still elusive, although biosynthesis of *t*-resveratrol in plants has been well-studied (*22*).

In conclusion, fungi were more effective in the elicitation of *t*-resveratrol and *t*-piceatannol in peanut calluses compared to the

commonly used abiotic stressors methyl jasmonate, salicylic acid, and sucrose. Reishi was applied to peanut callus in comparison with a peanut pathogen *B. theobromae* to understand the effect of Reishi on stilbenoid elicitation. Results showed the amounts of piceatannol and resveratrol induced by Reishi were comparable to those induced by the pathogenic one. Uptake of *G. lucidum* mycelium will additionally provide a variety of health-promoting effects.

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