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A New Method for Cultivation of Sclerotium of *Grifola umbellata*

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Sclerotia of *Grifola umbellata* were cultivated by two methods such as burying and root inoculation methods. The sclerotia of *G. umbellata* produced by the burying method were 6.0~6.8 × 3.4~4.6 × 1.8~1.9 cm (Width × Length × Thickness) in size and 17.3~19.6 g in weight, respectively. Their increase rate was 1.10~1.12 times. On the other hand, the sclerotia cultivated by the root inoculation method were 18.3~31.5 × 12.5~26.4 × 3.1~3.7 cm (W × L × T) in size and 219.1~576.6 g in weight, respectively. Their growth increment was 11.18~39.77 times. The rhizomorphs of *Armillaria mellea* were developed with a high density under fallen leaves layer covering cultivation site, and distributed mainly between soil surface and soil depth of about 10 cm as well as colonized prominently on the inoculated wood logs. Fungal interaction between *G. umbellata* and *A. mellea* were observed mainly in the stage of white sclerotium of *G. umbellata*. The sclerotia of *G. umbellata* which were developed newly and harvested in the root inoculation method were twined with root hairs of host tree and rhizomorphs of *A. mellea*. The sclerotia of *G. umbellata* decomposing root hairs of host tree were confirmed through SEM examination. Physiochemical characteristics of soil in all cultivation sites had no significant differences. Soil pH were in the range of pH 3.98~4.40. Organic matters were the range of 17.97~23.86% and moisture contents of soil were 12.00~18.20%. Soil temperatures showed 12.9~13.8°C in November and 22.0~23.9°C in August, respectively. In conclusion, the root inoculation method seems to be a practical method for cultivating sclerotia of *G. umbellata* due to its many advantages such as simplicity of inoculation process, shortening of cultivation periods and facility of harvest.

KEYWORDS: **Cultivation method, *Grifola umbellata*, Root inoculation, Sclerotium**

Grifola umbellata (Persoon : Fries) Pilát has been known as one of the medicinal fungi which belong to the family *Polyporaceae* of Basidiomycetes (Imazeki and Hongo, 1989). This fungus forms an underground irregular tuber such as sclerotium in its life cycle. The matured sclerotia of *G. umbellata* have been used for an important herbal medicine in Korea and China. Particularly, sclerotia of this fungus have been known to possess various medicinal effects such as a promotion of a diuretic process (Chen and Chen, 2000), an effect of hair regrowth (Inaoka *et al.*, 1994), a suppressive cytotoxic activity on leukemia (Ohsawa *et al.*, 1992), an inhibitory effect on urinary bladder cancer (Azuhata and Sugiyama, 1994; Yang, 1991), an antitumor activity and an immunopotentiation ability (You *et al.*, 1994). Based on the sclerotia of *G. umbellata*, the studies on the active substances such as ergosterol, ergosterol peroxide, glucan, sterol and polysaccharides have been carried out intensively by some researchers (Lu *et al.*, 1985; Miyazaki *et al.*, 1978, 1979; Ohta *et al.*, 1996a, b; Ueno *et al.*, 1980, 1982; Wei *et al.*, 1983). Besides, it was reported that extract from sclerotium of *G. umbellata* was not only outstanding in a cytotoxicity effect against human gastric cancer cell but also good in an antioxidant effect as well as antimicrobial activity against *Helicobacter pylori* which cause gastric and duodenal ulcer (Ha, 2001).

In spite of these medicinal properties, there was no report on the collection of natural sclerotium of *G. umbellata* and the artificial cultivation of its sclerotia in Korea. Therefore, we fully realized the necessity to perform mass cultivation of sclerotia of *G. umbellata*. This study was performed to develope a new method for mass production of sclerotia of *G. umbellata* and to shorten the period for cultivation of its sclerotia in field condition.

Materials and Methods

Isolates. Sclerotia of *G. umbellata* for inoculum sources were collected from Shan-xi Sheng, China in April, 1999. The collected sclerotia were maintained with sand and charcoal mixture (5 : 1, v/v) in semitransparent polypropylene bags containing moisture at 4°C and then used for inoculum sources. All isolates of *Armillaria* spp. were kept in potato dextrose agar (PDA) at 4°C. All isolates were listed in Table 1.

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Table 1. Fungal organisms used in this study

Species	Strain	Origin	Host	Fungal state
Grifola umbellata	DUM GU001	Shan-xi Sheng, China	–	Sclerotium
Armillaria mellea	DUM 007	Shan-xi Sheng, China	*Grifola umbellata*	Mycelia
A. mellea	DUM 003	IMP-CAMS[a], China	*G. umbellata*	Mycelia
A. tabescens	KFRI[b] 69126	Kimpo, Kyunggido	*Gastrodia elata*	Mycelia
Armillaria sp.	DUM 008	Pocheon, Kyunggido	*G. elata*	Mycelia

[a]IMP-CAMS; Institute of Medicinal Plant, Chinese Academy of Medical Science, Beijing, China.
[b]KFRI; Korea Forestry Research Institute, Seoul, Korea.

Table 2. Characteristics of cultivation methods to produce sclerotia of *Grifola umbellata*

	Site No.			
	1	2	3	4
Cultivation method	Root inoculation	Root inoculation	Burying	Burying
Location[a]	Mt. Woon-Gil	Mt. Woon-Gil	Mt. Woon-Gil	Mt. Nam
Size of cultivation site (Width × Depth × Length)	50 × 30 × 40 cm	50 × 30 × 40 cm	90 × 100 × 300 cm	90 × 100 × 300 cm
Inclination of cultivation area	30~40°	10~15°	20~30°	20~25°
Host trees	Chestnut tree (*Castanea crenata*)	Oak tree (*Quercus mongolica*)	None	None
Armillaria spp. used	*A. tabescens* KFRI 69126	*A. mellea* DUM 007	*A. mellea* DUM 003	*Armillaria* sp. DUM 008
Inoculation method of sclerotia	Attachment on lateral root of tree	Attachment on lateral root of tree	Attachment between wood logs	Attachment between wood logs
Burying depth[b]	15 cm	10 cm	25~30 cm	25~30 cm
Wood logs[c] Species	Maple and Oak tree	Maple and Oak tree	Chestnut, Maple and Oak tree	Chestnut, Maple and Oak tree
Size (Length × Diameter)	10~15 × 2~3 cm	10~15 × 2~3 cm	30 × 10~15 cm	30 × 10~15 cm
Quantity	5~10	5~10	40	40

[a]Mt. Woon-Gil is located in Namyangju, Kyunggido and Mt. Nam is located in Seoul.
[b]Burying depth is thickness of soil which covers cultivation site after inoculation.
[c]Wood logs were sterilized and added as a nutrient source for rhizomorphs growth of *A. mellea* in addition to those colonized favorably with rhizomorph of *A. mellea*.

Preparation of wood logs. Wood logs for inoculation were prepared with three species such as oak tree, chestnut tree and maple tree, and then cut into two types. One type was large wood logs that were cut into 30 × 10~15 cm (Length × Diameter) in size, whereas another was small wood logs and twigs that were cut into 10~15 × 2~3 cm (Length × Diameter) in size (Table 2). To colonize rhizomorph of *A. mellea*, large wood logs were wounded artificially with saw on their surface. After then, wood logs were soaked in water for 48 hrs. Large wood logs were put into semitransparent polypropylene bags (45 × 20 cm with 0.02 μm filter disc of 5 cm diameter) and autoclaved for 150 minutes at 121°C. Small wood logs and twigs were put into flask (2,000 m*l*), added with distilled water of 500 m*l*, and autoclaved for 60 minutes at 121°C. All sterilizations were done two times.

Culture of *Armillaria mellea*. To obtain inocula, *A. mellea* isolates from stock cultures were incubated for 1 month at 25°C in potato dextrose broth (PDB) and sawdust media (oak tree sawdust : rice bran : wheat = 6 : 2 : 2) plus 70% of moisture contents. To make colonization of rhizomorphs, PDB cultures were used as an inoculum for small wood logs and twigs, whereas sawdust cultures were used for large wood logs. Inoculated wood logs were kept for 2 months at 25°C in dark condition with 85% relative humidity.

Burying sclerotia of *G. umbellata* with wood logs. To produce sclerotia of *G. umbellata*, a cultivation site was digged out to the size of 90 × 100 × 300 cm (Width × Depth × Length) in the field. To promote the drainage of cultivation site, the bottom of the site was covered with

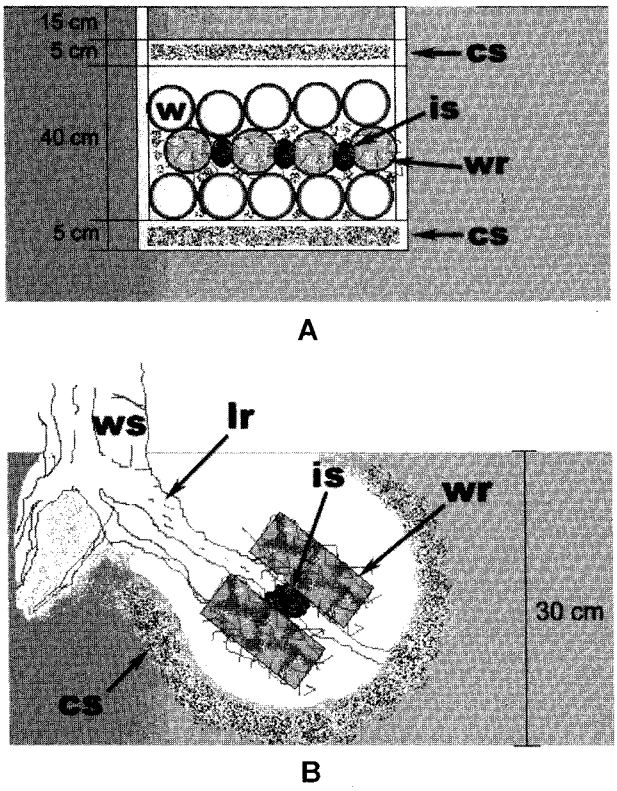

Fig. 1. Schematic features of cultivation methods for sclerotium of *Grifola umbellata*. **A**, Burying method. **B**, Root inoculation method. **cs**; sand and charcoal mixture (5 : 1, v/v), **is**; inoculated sclerotia of *G. umbellata*, **lr**; lateral root of host tree, **w**; sterilized wood logs for *Armillaria mellea* as a nutrient source, **wr**; wood logs colonized by rhizomorphs of *A. mellea*, **ws**; wood stump of host tree.

sand layer of about 5 cm. To exclude contaminants and control moistures in soil, charcoal was spread with about 3 cm on the sand layer. Large wood logs colonized by rhizomorphs of *A. mellea* were laid on the layer of sand and charcoal mixture with 3 cm apart from each wood log. Sclerotia were cut into small pieces of 5.8 × 2.3~4.8 × 2.0~2.3 cm (Width × Length × Thickness), two pieces of sclerotia per wood log were adhered between wood logs (Fig. 2B), and then filled with sand and charcoal mixture (5 : 1, v/v) in the vacancy between wood logs. With repetition of this way, the inoculation was conducted by three layers of wood logs. The inoculated wood logs were covered with sand and charcoal mixture (5 : 1, v/v) of about 5 cm, and then covered with soil layer of about 15 cm. Finally, the cultivation site was covered with fallen leaves of about 5 cm for protection of water vapor (Fig. 1A, 2B, Table 2).

Root inoculation. This method is more simple that *G. umbellata* and *A. mellea* were inoculated directly on the lateral root of host tree. Two kinds of host trees such as oak tree and chestnut tree which distributed on an incline plane were selected in this treatment. Also, sand and charcoal were used to promote drainage and prevent contaminants in soil. Holes for cultivating sclerotia were digged out to the size of 50 × 30 × 40 cm (Width × Depth × Length) in the circumstance of stumps of host trees, and then spread about 5 cm of sand and charcoal mixture (5 : 1, v/v) underneath cultivation site. Small pieces of sclerotia which were cut into 7.5~10.5 × 1.6~5.2 × 1.2~2.9 cm (Width × Length × Thickness) were adhered on the lateral root side with two small wood logs or twigs colonized by rhizomorphs of *A. mellea* and then fixed with gauze. After then the site was filled with sand and charcoal mixture (5 : 1, v/v) in the vacancy, and covered with soil layer of about 5 cm. To prevent water vapor and epipedons erosion from heavy rain, the surface of the site was pressed down and covered with fallen leaves and soil (Fig. 1B, 2A, Table 2).

Microscopic observation. To perform scanning electron microscopic examination, the collected samples were washed twice with 0.1 M sodium phosphate buffer (pH 7.2). The specimens were fixed overnight at 4°C in 2.5% glutaraldehyde, rinsed twice for 30 minutes in 0.1 M sodium phosphate buffer (pH 7.2) and post fixed at 4°C for 2 hours with 1% osmium tetroxide (OsO_4). The specimens double fixed were rinsed 3 times with 0.1 M sodium phosphate buffer (pH 7.2), and then dehydrated with a graded ethanol series such as 50%, 70%, 80%, 90% and 100%. The ethanol was substituted by isoamyl acetate. The specimens were dried by using a critical point dryer (Hitachi HCP-2, Japan), and then coated by using a sputter coater (Hitachi E-1010, Japan). As described on some reports (Choi *et al.*, 2002; Zarani and Christias, 1997), the specimens were examined under a scanning electron microscope (Hitachi S-2380N, Japan).

Physiochemical characteristic of soil in cultivation areas. To investigate soil characteristics of cultivation sites, soil samples were collected in August, 2000, and soil texture, pH, temperature, organic matters and moisture contents were investigated by using soil chemical analysis method (Jackson, 1958).

Results

Burying method. Inoculation of sclerotia of *G. umbellata* was performed at Mt. Woon-Gil, Namyangju, Kyunggido and Mt. Nam, Seoul in November, 1999 (Fig. 2B, Table 2). After 12 months of cultivation, sclerotia of *G. umbellata* were harvested from both sites. The size of the newly formed sclerotia with the exception of inocula was 6.0~6.8 × 3.4~4.6 × 1.8~1.9 cm (Width × Length × Thickness), their weight was 17.3~19.6 g and their increase rate was 1.10~1.12 times (Table 3). The rhizomorphs of *A.*

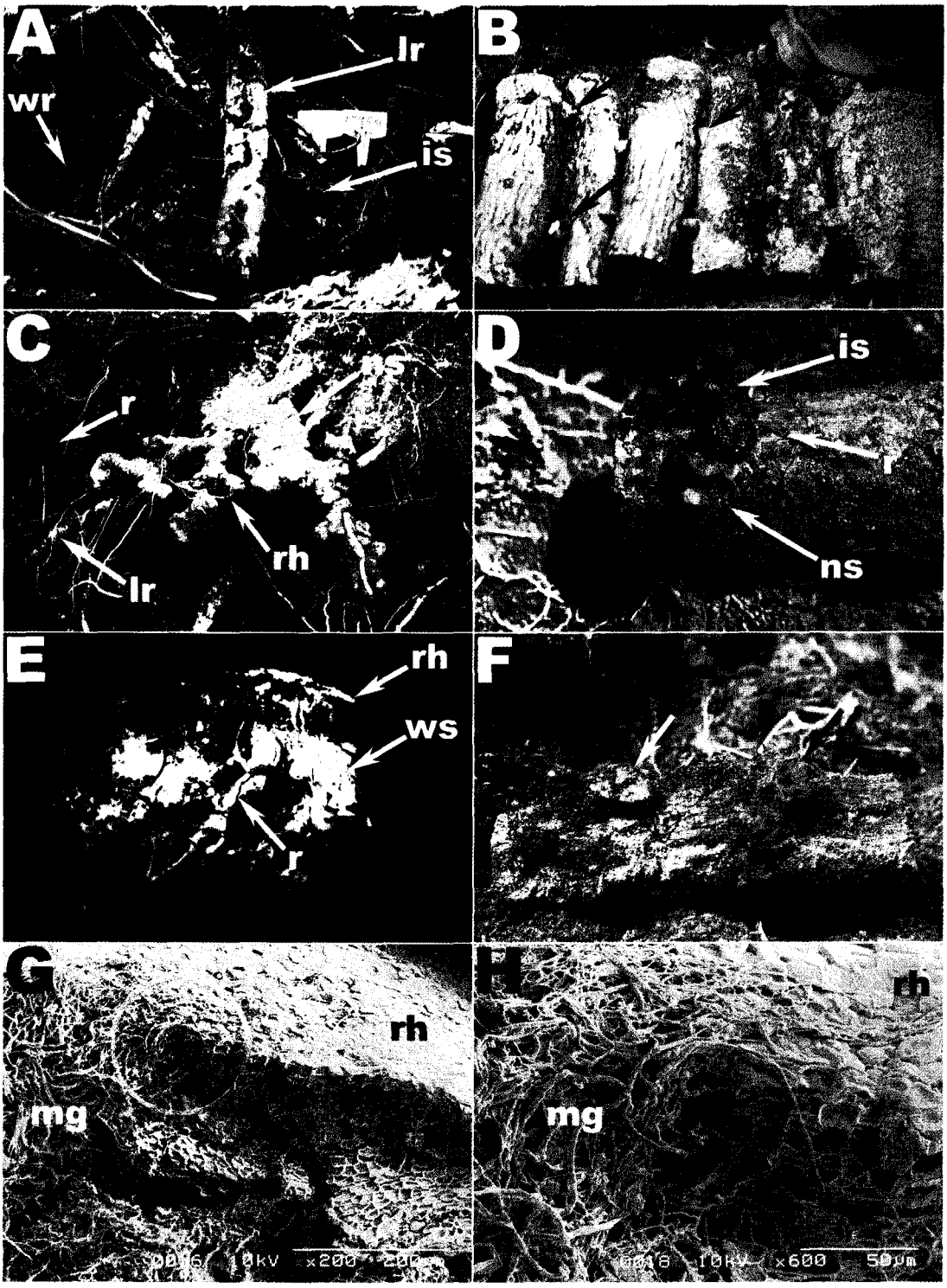

Fig. 2. Cultivation methods and sclerotia of *Grifola umbellata* produced by using the burying and root inoculation methods. **A**, Feature of the root inoculation method. **B**, Feature of burying method. Arrows indicate sclerotia as inocula. **C**, Sclerotium produced in oak tree (site No. 2). **D**, Sclerotia on the wood logs cultivated by the burying method. **E**, Feature of white sclerotium twined with root hair of host tree and rhizomorph of *A. mellea*. Circle represents mycelia of white sclerotium surrounding root hair of host tree which was observed by SEM and shown with figure G and H. **F**, Rotted sclerotia (arrows) of *G. umbellata* on the wood log colonized poorly with rhizomorph of *A. mellea* in the burying method. **G**, Mycelia of white sclerotium of *G. umbellata* surrounding and decomposing root hair tissue of host tree. **H**, Magnified feature of circle in figure G. **is**; inoculated sclerotium of *G. umbellata*, **lr**; lateral root of host tree, **mg**; mycelia of white sclerotium of *G. umbellata*, **ns**; new developed sclerotia of *G. umbellata*, **r**; rhizomorphs of *A. mellea*, **rh**; root hairs of host tree, **wr**; small wood logs colonized by rhizomorph of *A. mellea*, **ws**; white sclerotium of *G. umbellata*.

Table 3. The yield of sclerotia of *Grifola umbellata* harvested from each cultivation site

		Site No.			
		1	2	3	4
Cultivation method		Root inoculation	Root inoculation	Burying	Burying
Location[1]		Mt. Woon-Gil	Mt. Woon-Gil	Mt. Woon-Gil	Mt. Nam
Sclerotia inoculated	Quantity	2[a]	1[a]	2[b]	2[b]
	Size (cm)	10.5 × 5.2 × 2.9	7.5 × 1.6 × 1.2	5.8 × 4.8 × 2.3	5.8 × 2.3 × 2.0
	Weight (g)	19.6	14.5	17.5	15.7
Sclerotia harvested[2]	Quantity	1	1	2	1
	Size (cm)	18.3 × 12.5 × 3.1	31.5 × 26.4 × 3.7	6.8 × 4.6 × 1.9	6.0 × 3.4 × 1.8
	Weight (g)	219.1	576.6	19.6	17.3
Increase rate[3]		11.18	39.77	1.12	1.10
Rhizomorph development[4]		+++++	+++++	++	++

[1]Mt. Woon-Gil is located in Namyangju, Kyunggido and Mt. Nam is located in Seoul.
[2]The size and weight of the harvested sclerotium were measured with the exception of inoculated sclerotium.
[3]The increase rate represents weight increase of the harvest sclerotium.
[4]Development index of rhizomorphs of *A. mellea*: +++++; Rhizomorphs of *A. mellea* were developed with a high density on soil surface, colonized prominently on the inoculated wood logs and distributed throughout the cultivation site, ++; Rhizomorph of *A. mellea* were distributed mainly on the soil surface, colonized poorly on the inoculated wood logs and distributed sparsely throughout the cultivation site.
[a]The number of inoculated sclerotia totally in the cultivation site.
[b]The number of inoculated sclerotia per wood log.

mellea were developed mostly between soil surface and upper 10 cm of soil depth, and also colonized favorably on the wood logs of upper part in the cultivation site. In case that soil depth was more deep than about 30 cm from superficial layer, rhizomorph development was rarely observed and colonized poorly on the wood logs inoculated underneath ground of the cultivation sites. Particularly, it was obvious that the inoculated sclerotia of *G. umbellata* developed new fungal structure on the wood logs colonized favorably with rhizomorphs of *A. mellea* (Fig. 2D). On the other hand, the inoculated sclerotia were only adhered without any growth or with a rotted state on the wood logs developed poorly with rhizomorphs of *A. mellea* (Fig. 2F).

Root inoculation method. The sclerotial inoculation on root of host tree was performed at Mt. Woon-Gil in the same season as that of the burying method (Fig. 2A, Table 2). After 10 months of cultivation, sclerotia of *G. umbellata* were harvested from all cultivation sites. The produced sclerotia with the exclusion of inocula was 18.3~31.5 × 12.5~26.4 × 3.1~3.7 cm (Width × Length × Thickness) in size, their weight was 219.1~576.6 g and their yield increase was 11.18~39.77 times (Table 3). The harvested scleortia had 3 stages of sclerotial development such as white, grey and black sclerotia. In the early stage, rhizomorph of *A. mellea* invaded into white sclerotium of *G. umbellata*, and then both fungi were intermingled (Fig. 2E). In the second stage, grey sclerotium of *G. umbellata* digested the rhizomorph of *A. mellea* invading into sclerotial tissue and developed its sclerotial volume. In the final stage of sclerotial development of *G. umbellata*, rhizomorph of *A. mellea* did not invade into sclerotial tissue any more but colonized a superficial side of matured black sclerotium of *G. umbellata*. Newly developed sclerotia of *G. umbellata* were twined with root hairs of host tree (Fig. 2C, 2E). It was obvious under SEM examination that sclerotia of *G. umbellata* decomposed root hairs of host tree (Fig. 2G, 2H). Generally, the rhizomorphs of *A. mellea* were developed with a high density between

Table 4. Physiochemical characteristics of soil in cultivation sites

	Site No.			
	1	2	3	4
Location[a]	Mt. Woon-Gil	Mt. Woon-Gil	Mt. Woon-Gil	Mt. Nam
Soil texture	Clay loam	Silt clay loam	Clay loam	Sandy loam
pH	4.40	4.31	4.27	3.98
Organic matter (%)	22.63	22.06	23.86	17.97
Moisture contents (%)	17.38	16.51	18.20	12.00
Soil temperature on November (°C)	12.9	13.0	13.2	13.8
Soil temperature on August (°C)	23.4	22.0	23.9	23.3

[a]Mt. Woon-Gil is located in Namyangju, Kyunggido and Mt. Nam is located in Seoul.

soil surface and around 10 cm of soil depth in the cultivation site, and also colonized favorably on the inoculated wood logs. Particularly, rhizomorphs of *A. mellea* were distributed prominently on the soil surface of the site No. 2 in which the biggest sclerotium of *G. umbellata* was produced (Fig. 2C, Table 3). This fungal structure grew out of the soil surface and just below fallen leaves layer covering the cultivation site. Although it was difficult to distinguish differences of sclerotial production which were related to host trees and isolates of *Armillaria* spp., the rotted sclerotia were not collected in this method. Also, the root disease of the host trees caused by *A. mellea* and *G. umbellata* was not observed in treatment of root inoculation.

Physiochemical characteristics of soil in cultivation sites. To investigate soil characteristic of cultivation sites, soil samples were collected with 5 times of each 100 g. Soil textures showed clay loam, silt clay loam and sandy loam, respectively. Soil pH were in the range of pH 3.98~4.40. Organic matters and moisture contents of soil were 17.97~23.86% and 12.00~18.20%, respectively. Soil temperatures were shown as 12.9~13.8°C in November and 22.0~23.9°C in August (Table 4).

Discussion

The natural habitat of *G. umbellata* is on the ground, arises from dead roots or buried wood on stumps, and prefers birch, maple, beech and oak trees. *G. umbellata* has been distributed in China, Japan, and temperate regions of the Northern Hemisphere (Chen and Chen, 2000; Lee, 1988). Particularly, the sclerotial development of *G. umbellata* is associated closely with rhizomorphs of *A. mellea*. Xu and Guo (1992) reported that sclerotium of *G. umbellata* had a symbiotic relationship with rhizomorph of *A. mellea*. The rhizomorph of *A. mellea* adhered and invaded into sclerotia of *G. umbellata* (Guo and Xu, 1993a, b), and then fungal mass of rhizomorph of *A. mellea* was digested by sclerotia of *G. umbellata* as a nutrient source (Guo and Xu, 1992). In this respect, rhizomorph of *A. mellea* is crucially required to develop sclerotia and also produces matured sclerotia of *G. umbellata* in nature. *A. mellea* capable of causing pathogenicity is noted for its rhizomorphs and causes a serious root rot disease of tree in a forest and orchards (Raabe, 1972; Rizzo *et al.*, 1998). Also, *A. mellea* has been studied assiduously due to its symbiotic association with certain orchid plant such as *Gastrodia elata* (Kim *et al.*, 2000; Kusano, 1911). The rhizomorphs of this fungus are distributed in the nearer soil surface such as the range of 2.5~10 cm and are rarely found below 30 cm in a soil profile even though roots of stumps colonized by *A. mellea* are present below that depth (Redfern, 1973). Morrison (1976) reported that the rhizomorph growth of *A. mellea* was not related with a soil type or texture but influenced by moisture contents, oxygen concentration and nutrients in soil. Therefore, for mass production of sclerotia of *G. umbellata*, the cultivation method should be considered simultaneously on the optimal condition to develop rhizomorph of *A. mellea* and growth environment of sclerotia of *G. umbellata* as well as symbiotic relationship between two fungi.

In this research, two types of cultivation methods were performed in the field and sclerotia of *G. umbellata* were harvested in all cultivation sites after 10~12 months. The yield, quantity and the commercial value of the sclerotia produced by the root inoculation method were excellent and promising comparing to those produced by the burying method (Table 3). Of all cultivation sites, physiochemical characteristics of soil did not show a wide difference between the burying method and the root inoculation method (Table 4). Therefore, it is supposed that sclerotial production has close relation with burying depth and rhizomorph development of *A. mellea* rather than physiochemical characteristics of soil environment.

In the burying method, the size and weight of the produced sclerotia were similar to those of the sclerotia inoculated before 12 months (Fig. 2D, Table 3). Although inocula developed new sclerotia in burying treatment, it seemed that inoculated sclerotia did not secure sufficient space for their growth between wood logs. Rhizomorphs of *A. mellea* distributed mainly on soil surface or colonized thinly on the inoculated wood logs. Particularly, a number of sclerotia inoculated were rotted or adhered in the form of ceasing their growth on the wood logs. In these results, it seemed that burying depth of 30~50 cm was too deep to develop sclerotia of *G. umbellata* and rhizomorph of *A. mellea*.

On the other hand, the sclerotia of *G. umbellata* with high commercial value were harvested by using the root inoculation method. The weight of the produced sclerotia was about 7~40 times as much as that of the inoculated sclerotia in this treatment. The harvested sclerotia showed three stages of sclerotial development such as white, grey and black sclerotia in root inoculation method. In this respect, the sclerotia cultivated by the root inoculation method seemed to develop more briskly than those produced by burying method. Sclerotia of *G. umbellata* adhered stably on the lateral root of host tree and decomposed root hairs of host tree (Fig. 2G, 2H) as well as rhizomorphs of *A. mellea*. The largest sclerotium produced in oak tree (Site No. 2) grew to upward direction on the soil surface and just below fallen leaves layer covering the cultivation site (Fig. 2C, Table 2). Therefore, it supposes that *G. umbellata* prefers aerobic condition, and burying depth of inoculum is very important to cultivate sclerotia of *G. umbellata*. According to Morrison (1976), the rhizomorph growth of *A. mellea* was along gradients of

increasing oxygen and decreasing carbon dioxide concentrations. Because burying depth of the root inoculation method is shallow, this method seems to be probable to offer aerobic condition and enough space for inducing sclerotial development of *G. umbellata* and rhizomorph growth of *A. mellea*. In addition, sclerotia of *G. umbellata* and rhizomorph of *A. mellea* are possible to secure plentiful nutrient sources such as fallen leaves and root exudates from lateral root of host tree. Besides, fallen leaves layer will be helpful to keep temperature and moisture contents of cultivation site. There was no a difference of rhizomorph development on soil surface in all cultivation sites. It seemed that the number of wood logs or their size was not affective to the yield of sclerotia, and so small wood logs and twigs are sufficient as inoculum sources for culturing *A. mellea*. In regarding growth environment of sclerotia of *G. umbellata*, rhizomorph development of *A. mellea* and symbiotic association between *G. umbellata* and *A. mellea*, root inoculation method seems more profitable than burying method. In conclusion, the root inoculation method seems to be an ideal method for cultivating sclerotia of *G. umbellata* due to its many advantages such as simplicity of inoculation process, shortening of cultivation periods and facility of harvest.

In the present study, *A. tabescens* used for *G. elata* produced new sclerotia of *G. umbellata*. This result presents the possibility of dual cultivation of *G. umbellata* and *G. elata* at the same place. Furthermore, it is presumed that *G. umbellata* has the probability of symbiotic relationship with other *Armillaria* spp. besides *A. mellea* reported up to now. Therefore, the symbiotic relationship between *G. umbellata* and *A. mellea* should be examined more precisely through various approaches.

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