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Cultivation of *Ganoderma* Bonsai

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ABSTRACT: *Ganoderma lucidum* is not only the leading medicinal mushroom in China, but it has long been valued for the aesthetic beauty of its fruiting body. The methodology of preparation of the substrate and manipulation of the morphogenetic factors to produce *Ganoderma* Bonsai will be described. Strain selection and examples of various *Ganoderma* Bonsai forms will also be given for this woody mushroom with a history in the arts as a symbol of life fulfillment and longevity in China.

1 INTRODUCTION

Fruiting bodies of *Ganoderma lucidum* have long been featured in Chinese art and are recognized as a symbol of longevity. *Ganoderma* Bonsai have recently become very popular as an art form. The artistic concept of what makes an interesting *Ganoderma* Bonsai and the scientific knowledge of how to cultivate the *Ganoderma* mushroom involving aseptic techniques are both important in creating an impressive *Ganoderma* Bonsai.

2 MATERIALS AND METHODS

2.1 *Strain isolation and selection*

This paper focuses on *G. lucidum* strains but also includes other *Ganoderma* species. Most *G. lucidum* strains were isolated from North Eastern and South Eastern United States by: 1) tissue culture from the whitish growing edge of the basidiocarp, or of the immature basidiocarp, 2) tissue culture from the context (which contains a higher proportion of

viable generative hyphae) of the mature basidiocarp without whitish growing edge, 3) inoculation using the host-plant tissue adjacent to the *Ganoderma* fruiting body, immediately following the removal of the highly contaminated old *Ganoderma* basidiocarp from the host plant, or by 4) germination of *Ganoderma* basidiospores collected at the onset of sporulation period (Chen and Hu 1995).

Tissue cultures were made from minute pieces of fungal inner tissue taken aseptically from the basidiocarp with a sharp, thin scalpel and inoculated onto plates or agar slants of water agar, PDA (potato dextrose agar), or PDA with antibiotics. Alcohol surface sterilization was used when necessary. Fruiting potential, structured characteristics of the basidiocarp, branching sensitivity of the stipe, secretion of the laccate substance, and coloration of the fruiting body were used as selection criteria for strains of *G. lucidum* in Bonsai cultivation.

2.2 *Substrate formulation for basidiocarp production*

Supplemented sawdust-bran substrate of the following formulation was used: oak sawdust 80%, coarse unprocessed wheat bran 18%, suplements of sucrose 1%, and calcium, either as calcium carbonate or calcium sulfate, 1%. The water content was approximately 67%. For 500 g dry substrate (containing 400 g oak sawdust and 90 g wheat bran), 1 liter of distilled water containing sucrose and $CaCO_3$ was added (5 g each).

2.3 *Spawn run*

Liquid spawn was used to inoculate the suplemented sawdust-bran substrate. The following protocol was used: 1) fungal mats of *G. lucidum* were harvested from a 4 to 7 day old liquid culture (20 ml PDB/ 250 ml Erlenmeyer flask), incubated without agitation at 27 to 30°C in the absence of light, 2) the mats were homogenized twice for 3 seconds each at high speed in a blender, 3) the macerate was inoculated onto the substrate in Bonsai or Ikebana containers previously sterilized at 121°C for 20 min inside of loosely fitting sterile polypropylene bags (Unicorn), and 4) the inoculated synthetic logs were incubated at 27 to 30°C in darkness for 2 to 3 weeks or until the substrate was thoroughly penetrated by the mycelium.

Alternatively, pileate *Ganoderma* fruiting bodies were cultivated in the polypropylene bag first and then embedded in the chosen container. The fruiting culture within the bag was then covered with moss sod or lichens simply to disguise the bag in an interesting Oriental pottery or china container.

2.4 *Initiation of Ganoderma primordia*

Following a vigorous spawn run in the absence of light, the cultures were exposed to subdued light (150-200 lux) to trigger primordial formation. The localities for primordial initiation could be directed to a certain extent by selective exposure of the spawn to light and air. Extra primordia were removed by pruning.

2.5 *Cold shock as a re-inforcement of primordial initiation*

Occasionally unresponsive spawns were subjected to a cold shock by leaving the synthetic logs at 0°C or slightly warmer in the freezer compartment of a home refrigerator overnight (10 to 12 hr or longer). Primordia usually were formed after 1 to 2 weeks or sometimes for a longer period depending on the amount of the substrate and the severity of the cold shock. Primordia were not observed when the temperature of the cold shock was too high. A second cold shock could then be applied.

2.6 *Casing*

Following primordial formation, the polypropylene bag was removed. A layer of casing of moss sod was used, leaving the primordia protruding above the moss casing. To maintain the proper moisture level, water was sprayed onto the culture only when neccessary. Replacement of the casing was made when the moss turned brown.

2.7 *Manipulation of the physical parameters for Bonsai formation*

Carbon dioxide level was a key morphogenetic factor for controlling branching of *Ganoderma* stipes. The level of CO_2 was raised from 0.03% in the normal air to 0.1 to 1% to facilitate branching in creating antler-form bonsai, or pileate bonsai with branched stipes. The level of CO_2 was raised simply by restriction of air circulation (ventilation) in an enclosed synthetic bag.

Another vital parameter in shaping the *Ganoderma* Bonsai is the direction of the light source in relation to the culture based on the phototropic response of the *Ganoderma* fruiting body. Periodic rotation of the culture in relation to the light source was determined by the angle of the stipe and its desired future orientation in shaping an artistic Bonsai.

Inoculated *Ganoderma* synthetic logs were maintained at 27 to 30°C or at room temperature, and 70 to 80% R.H. for antler-form Bonsai. In contrast, the relative humidity was raised to 85 to 95% under proper

air circulation for the formation of pileate *Ganoderma* Bonsai. To produce firm specimens, the R.H.was lowered to 50 to 60% at a later stage, even following the maturation of the fruiting bodies in some cases. The concept of an artistic Bonsai or Ikebana in plants served as a model for aesthetic *Ganoderma* Bonsai.

3 RESULTS

3.1 *Substrate formulation and cultivation of Ganoderma basidiocarps*

Formulation of the suplemented sawdust-bran substrate and the process of producing *Ganoderma* fruiting bodies were first tested on a laboratory scale with positive results. High humidty (85 to 95% R.H.) and proper air circulation are crucial for the formation of pileated fruiting bodies under subdued light (150 to 200 lux). The laboratory work was first sucessfully scaled up by a Canadian grower using synthetic bags (Sonnenamn, E., personal communication).

3.2 *Reinforcement by cold shock for primordial initiation*

Spawns which did not respond to the standard treatment of primordial initiation by exposure to light and air were subjected to a cold shock. The cold shock as described in materials and methods was effective in repeated experiments in triggering the formation of *Ganoderma* primordia. The *Ganoderma* Bonsai in Fig. 1 is an example.

3.3 *Effect of physical parameters on basidiocarp formation*

Growth of stipes at different temperatures showed that thicker stipes were formed at lower temperatures at a slower rate. Colder temperatures (20 to 25°C or lower) were therefore used at the initial stage of stipe development to produce a thicker and stronger base for antler Bonsai. Preliminary investigations showed that under intense lighting or in the absence of light, formation of pigment and the laccate substance was inhibited. Subdued lighting (150 to 200 lux) was therefore used for the development of *Ganoderma* basidiocarps. Branching of stipes was achieved by raising the level of carbon dioxide, while the length of the stipe was enhanced by dim light. Orientation of the light source was effective in shaping the direction and angle of stipe formation in Bonsai.

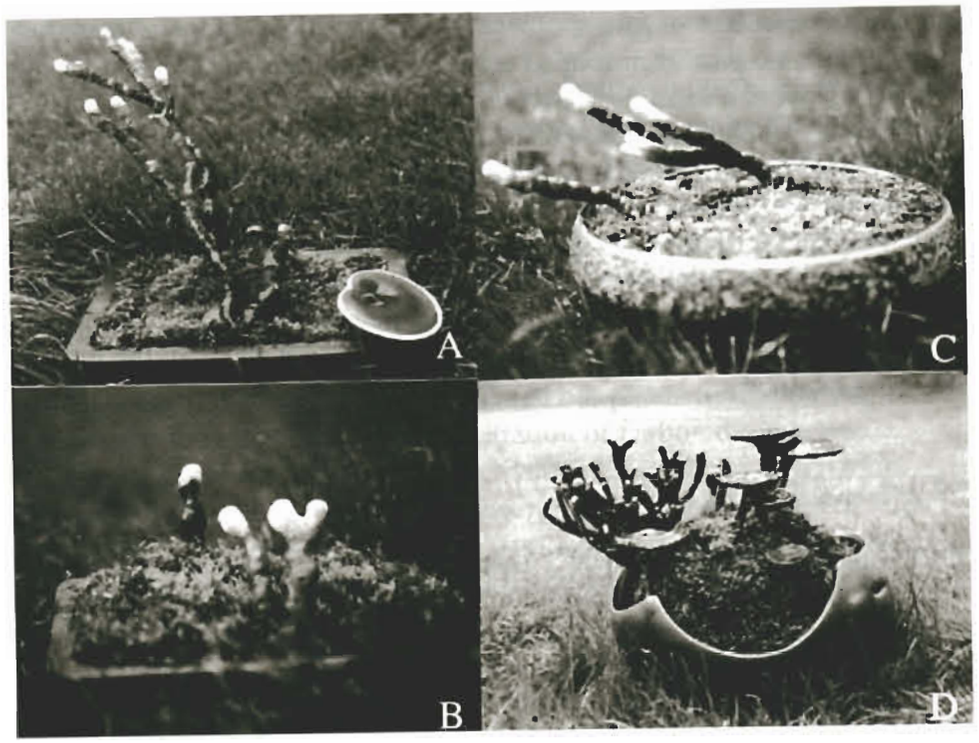

Figure 1. Antler and pileate *Ganoderma* Bonsai

3.4 *Variation of basidiocarp characteristics and selection of strains*

Among *G. lucidum* strains and other *Ganoderma* species tested, variation in the production of a shiny laccate substance and coloration was observed. Strains particularly sensitive to carbon dioxide in branching were also noted. Differences in basidiocarp characteristics, in shape, thickness, and size, were found. The following *G. lucidum* strains were noted with great interest: Strain 9260 isolated from oak in Athens, GA, is highly laccate and has double ramhorn shaped basidiocarps. Strain 9264, originated from Korea, is also highly laccate but has unusually small caps. Strain 9256, another strain isolated in Athens, GA from red maple has an unusual deep color. As a promising candidate for antler-form Bonsai, Strain 9267 was shown to be sensitive to carbon dioxide in branching. Highly laccate strains with good color and interesting basidiocarps were selected for pileate Bonsai formation, while a strain demonstratin sensitivity to carbon dioxide and light was selected for creating antler-shaped Bonsai.

3.5 *Ganoderma Bonsai forms*

Fig. 1 A, B and C show antler *Ganoderma* Bonsai cultivated by using strain 9267. The pileate *Ganoderma* Bonsai in Fig. 1D is an American strain 9260 first cultivated in a polypropylene bag before being embedded in an interesting Japanese Ikebana container. It was demonstrated experimentally that the size of basidiocarps can be controlled by maintaining a certain number of primordia per culture. For instance, larger basidiocarps were developed from one or two primordia per bag by pruning the extra primordia, while smaller fruiting bodies were formed when all the primordia were retained.

4 DISCUSSION

Following our cultivation practice based on prescribed substrate formulation, a Canadian grower (Sonnenamm) succeeded in scaling up production. Well developed pileate fruiting bodies of *G. lucidum* in synthetic bags were obtained. Others who succeeded in cultivation of *G. lucidum* adapted similar processes and formulation of substrates containing lignin (Cha 1991, Chang 1983, Hseu 1993, Liu *et al.* 1990, Lu and Chang 1975, Quimio 1986, Stamets 1993, Tong and Chen 1990 and Triratana *et al.* 1991).

Sawdust, a major component of the substrate, cannot be dissolved in water. It is therefore difficult to achieve a homogeneous mixture. The moisture content, sawdust particle size, and the packing of the substrate affect aeration and the nutrient transport in the medium. Adjustment of water content may be required depending on the size of the sawdust and its moisture content.

Attempts can be made to direct the spacing of primordia. The spawn can be covered to prevent light exposure except at spots where primordia are anticipated. The bag can also be punctured at specific localities. The punctured bag can be loosely fitted inside of another larger sterile bag to cut down the possibility of contamination.

Growers often experience that not all spawns respond to the standard treatment for primordial initiation by exposure to light and air. Cold shock can be applied to such nonresponsive samples as a stimulus. It is theorized that autolysis occurs in some of the mycelium as a consequence of the cold shock. Autolytic products from the damaged fungal tissue lead to stimulation of primordial initiation.

New growers who obtained only antler basidiocarps find that this is usually due to the accumulation of carbon dioxide produced from the respiration of massive mycelial growth under suboptimal air circulation

and insufficient humidity. Knowing how to produce both antler and pileate *Ganoderma* basidiocarps can be helpful in Bonsai creation.

Ganoderma Bonsai are highly valued in China. Stamets (1993) included an antler *Ganoderma* Bonsai displayed in a Chinese mushroom museum in his new book. Strains sensitive to carbon dioxide are ideal for developing antler Bonsai or pileate Bonsai with interesting branching. Hseu (1993) used sensitive strains of *G. tsugae* for the production of antler fruiting bodies. Lu and Chang (1975) studied the effect of carbon dioxide concentration on stipe and cap formation in *G. lucidum*. Of great interest is their finding that by raising the air carbon dioxide level to 0.1 to 1%, branching of stipes was stimulated but pileus formation was inhibited. However, when the high concentration of carbon dioxide was removed, pileate formation was re-activated. To cultivate antler-form Bonsai, colder temperature is more desirable at the initial stage of stipe formation. A thicker base of the stipe formed at a lower temperature is more suitible for division into secondary and tertiary branches. It is also a stronger Bonsai foundation.

Not only can the stipes be manipulated into different thickness, length, or orientation, but the fruiting bodies can also be regulated in terms of size, orientation and habit of growth (fused or distinct from each other). By controlling light, the presence or absence of color and laccase secretion can be regulated. Such manipulation has been applied to the formation of bird-like *Ganoderma* Bonsai which is popular in China (Zhu 1994). Highly laccate strains which produce beautiful shiny fruiting bodies are aesthetically interesting for the creation of *Ganoderma* Bonsai. Color is another attraction.

Diversity in shape, size, color and production of laccate substance of *Ganoderma* basidiocarps makes it interesting to create an assortment of *Ganoderma* Bonsai. Strains sensitive to carbon dioxide and light can also be selected to create antler-form Bonsai. Growers of *Ganoderma* Bonsai would be wise to acquire a pool of potentially desirable strains in experimenting with Bonsai formation.

Samples of live *Ganoderma* Bonsai are by far the most interesting. The Bonsai can be kept alive under refrigeration for extended periods of time as long as it is kept from dehydrating. When the Bonsai dries out, it can still be preserved because of the polypore mushroom's woody nature. Storage of the dried *Ganoderma* fruiting bodies should be protected from insect infestation. Miniature beetles known as ciids (Ciidae family) can demolish dried *Ganoderma* fruiting bodies into powder (Madenjian *et al.* 1993). Field materials should be kept away from the dried specimens.

In addition to the scientific knowledge for producing *Ganoderma* fruiting bodies, the artistic concept of Bonsai formation is important.

Bonsai plants and Ikebana arrangements are valuable models for *Ganoderma* Bonsai. Branching and formation of the stipes can be either slanted upward or cascaded downward. Simplicity and asymmetrical beauty are two of the guiding principles in Bonsai formation.

According to the ancient literature in China (Wasson 1976), a legendary encounter of an intricate *Ganoderma* mushroom with nine caps on a highly branched stipe was recorded during the Han Dynasty. The legendary discovery took place in the courtyard of the palace of Han Wu Di, a well-known emperor in the Han Dynasty. The palace was under the construction at the time. Presumably, accumulation of sawdust occurred as a result of the construction work, and sawdust could serve as a substrate for the growth of *Ganoderma*. The finding of the rare mushroom led to celebration and festivity on a grand scale. Treasured as a divine mushroom, *G. lucidum* was a symbol of good fortune and longevity. Although creation of *Ganoderma* Bonsai can be labor intensive, this precious *Ganoderma* mushroom of legend, whether real or fictious, remains today an inspiration for the creation of *Ganoderma* Bonsai.

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