
Morphological variations among sporophores of *Ganoderma lucidum*

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Pure cultures of three *Ganoderma lucidum* isolates were used to study various morphological variations occurring within the sporophores. Variations were found in almost all the morphological characteristics studied. The colour of upper pilear surface varied among isolates and was deep red in G-1, yellowish brown in G-2 and reddish brown in G-3. The colour of context tissue of basidiocarp varied from white in G-1 and G-2 to light brown in G-3. Number of pores per millimetre varied from 5-8 in G-1, 5-7 in G-2 and 4-8 in G-3. Sporophore shapes in isolate G-1 were fan, kidney, antler and circular while in G-2 and G-3 these were fan and antler shaped. The attachment pattern found in isolate G-1 was lateral and divided, in G-2 it was lateral, central and divided and in G-3 it was lateral and central. The margin pattern observed in isolate G-1 and G-2 was smooth and shallow lobed while isolate G-3 had smooth, shallow lobed and deep lobed margin patterns. The surface pattern in isolate G-1 and G-3 was observed as smooth and shallow waved while in G-2 it was smooth, shallow waved and deep waved. The colour of stipe varied from deep brown in G-1 and G-3 while in G-2 it was brown coloured. The stipe also appeared laccate in all the specimens. The average thickness of stipe ranged from 1.6 cm in G-1 to 2.2 cm in G-2 and 1.4 cm in G-3. The length of stipe varied from 7.5 cm in G-1 to 8.5 cm in G-2 and 10.5 cm in G-3.

Key words: Basidiocarps, *Ganoderma lucidum*, pileus, stipe.

INTRODUCTION

Ganoderma was established by Karsten (1881) with *G. lucidum* (W. Curt.:Fr.) P. Karst. as the only species. *Ganoderma* is a morphologically complex genus belonging to family Ganodermataceae of order Aphyllophorales under which about three hundred species, sub species and varieties have been published (Ryvarden, 1994 ; Moncalvo *et al.*, 1995). Because of taxonomically different view point among collectors, the taxonomy of these fungi is very subjective and confused. The prevailing disagreement in the classification of polypores results from multiplicity of criteria on which several systems of classification are based. Murril (1902) considered primary taxonomic characters to be host specificity, geographical distribution and mac-

romorphology of fruiting body while Nobles (1958) suggested use of cultural characters in developing taxonomy of Polyporaceae that reflects natural relationships and phylogeny. Corner (1983) has mentioned that taxonomy of Ganodermataceae should be simplified to base a new classification on basidiocarp developmental studies in the field. Gilbertson and Ryvarden (1986) reported microscopic characters as of major taxonomic importance in polypores. Recently Tiwari *et al.* (2005) reported utility of different classical and modern taxonomic aspects of genus *Ganoderma* including macro and micro morphology, cultural characters and phylogeny in relation to Indian species. Thus an attempt was made to study the morphological variations among sporophores of *G. lucidum* which could be of help in its taxonomical clarification.

MATERIALS AND METHODS

Cultures and maintenance

Pure cultures of three isolates of *G. lucidum* used in present investigation were coded as G-1, G-2 and G-3. Pure cultures of G-1 (Israeli isolate or NRCM - OE- 62) and G-2 (NRCM -OE-52) were procured from mushroom centre of Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan and Directorate of Mushroom Research, Solan, H.P, respectively, while G-3 was a local isolate the pure culture of which was made through tissue culture from basidiocarp growing on shisham tree in Chatha area of Jammu, J&K. The pure cultures of *G. lucidum* isolates were maintained by repeated sub culturing on malt extract agar MEA at 10 days interval and incubated at $25\pm 1^\circ\text{C}$ temperature.

Spawn preparation

The spawn of these three isolates was prepared separately using culture bits from pure culture of these isolates cultured on malt extract agar. Wheat grain based spawn used here was prepared as per the method suggested by Munjal (1973). The boiled and soaked wheat grains were mixed with 2 per cent calcium sulphate and 6 per cent calcium carbonate on wet weight basis of grains. About 250 grams of these grains were filled in empty half liter glass bottles, plugged with non absorbent cotton and autoclaved at 20 lb psi pressure for two hours. Each bottle after cooling was shaken to remove clumps and inoculated separately with 10 days old culture bits of *G. lucidum* isolates under aseptic conditions. At least 10 spawn bottles of each isolate were prepared. The bottles were then incubated at $30\pm 1^\circ\text{C}$ till full spawn run and served as mother spawn.

Preparation of substrates

Four basal substrates used in the study were shisham (*Dalbergia sissoo*) sawdust, sugarcane bagasse, wheat straw and paddy straw. The substrates were moistened appropriately to give moisture content of 65 per cent. All the substrates were amended with wheat bran (20%) and CaCO_3 (2%). The substrates were filled in autoclavable polypropylene bags (50x35 cm) and plugged with non absorbent cotton after putting a plastic ring in the neck. The substrates were autoclaved at 15 psi

pressure for 2 hours. After cooling the bags were aseptically inoculated with wheat grain spawn @ 6 per cent on wet weight basis. The test isolates were grown separately on each substrate and for each treatment 8 bags were inoculated. Thus a total of 96 bags were used. The inoculated bags were kept in dark room for spawn run where temperature was around $30\pm 2^\circ\text{C}$ and humidity was maintained at 80 ± 5 per cent. After complete spawn run the bags were given slits by blade, kept in cropping room with diffused light and humidity was maintained at 90-95 per cent till primordial initiation. After primordial initiation humidity was reduced to 80 ± 5 per cent. During development and maturation of sporophores humidity was further reduced to 70 ± 5 per cent and good ventilation and diffused light maintained in cropping room.

Observation of variability among sporophores

Various morphological variations occurring in the sporophores were recorded from immature to fully mature stages.

RESULTS AND DISCUSSION

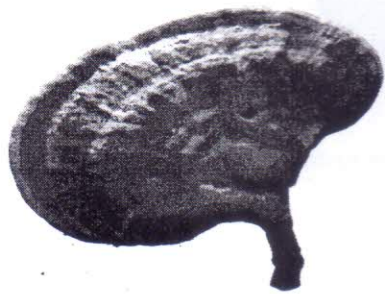
Pileus characteristics

The colour of upper pilear surface of mature basidiocarps was deep red in G-1, yellowish brown in G-2 and reddish brown in G-3. However, the pilear surface was laccate in all the isolates studied. The pilear margin appeared white in immature basidiocarps and disappeared in mature ones. The colour of context tissue of mature basidiocarps varied from white in G-1 and G-2 to light brown in G-3. Number of pores per millimetre of mature basidiocarps varied from 5-8 in G-1, 5-7 in G-2 and 4-8 in G-3. Average pileus diameter of mature basidiocarps varied from 11.8 cm in G-1, 10.6 cm in G-2 and 10.1 cm in G-3.

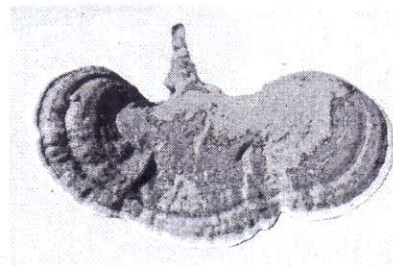
Morphological differences were observed in the shapes of mature basidiocarps. Sporophore shapes in isolate G-1 were fan (Fig-1 a), kidney (Fig-1 b), antler (Fig-1c) and circular (Fig-1d) while in G-2 and G-3 fan and antler shapes were observed. The attachment pattern found in isolate G-1 was lateral (Fig-2 a) and divided (Fig-2 c), in G-2 it was lateral, central (Fig-2 b) and divided and in G-3 it was lateral and central. The margin pattern observed in isolate G-1 and G-2 was smooth (Fig-3 a) and shallow lobed (Fig-3 b) while

Table 1: Morphological characteristics of sporophores of *G. lucidum*

Sporophore characteristic	Isolate		
	G-1	G-2	G-3
1. Pileus			
a) Colour	Deep Red	Yellowish Brown	Reddish Brown
b) Margin colour	White	White	White
c) Context colour	White	White	Light brown
d) Shape	Fan, Kidney , Antler and Circular	Antler and fan	Antler and Fan
e) Average diameter	11.8 cm	10.6 cm	10.1 cm
f) Laccate/ non laccate	Laccate	laccate	Laccate
g) stipitate/ sessile	Stipitate	Stipitate	Stipitate
h) Attachment pattern	Lateral and divided	Lateral, divided and central	Lateral and central
i) Margin pattern	Smooth and shallow lobed	Smooth and shallow lobed	Smooth, shallow lobed and deep lobed
j) Surface pattern	Smooth and shallow waved	Smooth, shallow waved and deep waved	Smooth and shallow waved
2. Stipe			
a) Average thickness	1.6 cm	2.2 cm	1.4 cm
b) Colour	Deep Brown	Brown	Deep Brown
c) Average Length	7.0 cm	8.5cm	10.5 cm
d) Laccate/non laccate	Laccate	Laccate	Laccate



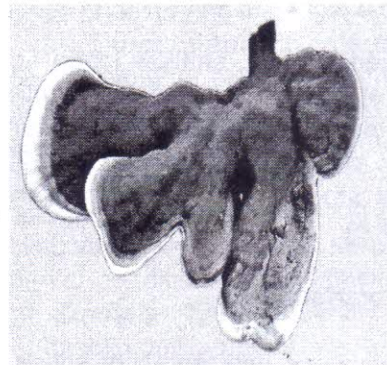
1 a: Fan shaped



1 b: Kidney shaped

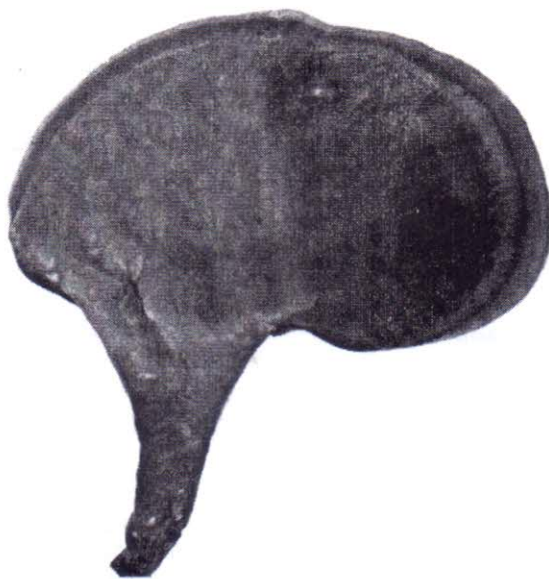


1 c: Circular shaped

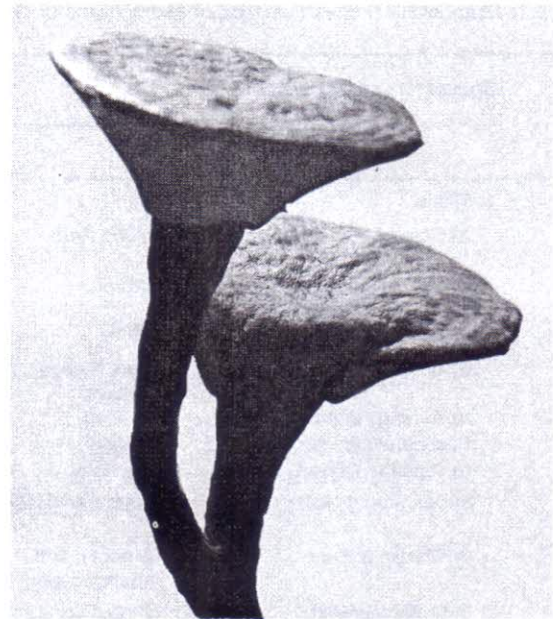


1 d: Antler shaped

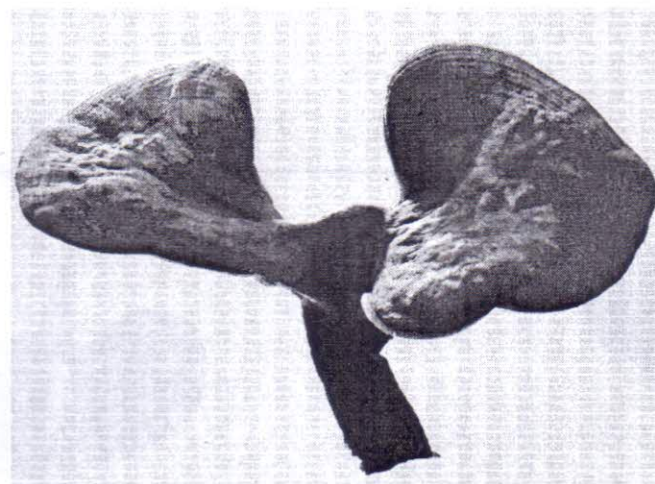
Fig. 1. (a-d). Sporophore shapes in *Ganoderma lucidum*



2 a: Lateral attachment



2 b: Central attachment



2 c: Divided attachment

Fig. 2. (a-c). Attachment patterns in *Ganoderma lucidum*

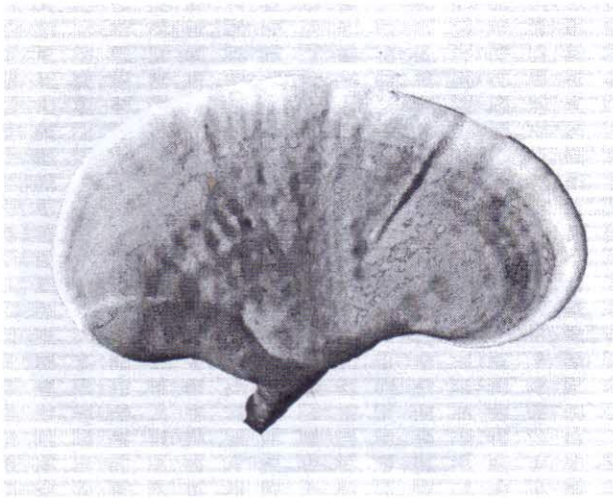
isolate G-3 had smooth, shallow lobed and deep lobed (Fig-3 c) margin patterns. The surface pattern in isolates G-1 and G-3 was observed as smooth (Fig-4 a) and shallow waved (Fig-4 b) while in G-2 it was smooth, shallow waved and deep waved (Fig-4 c).

Stipe characteristics

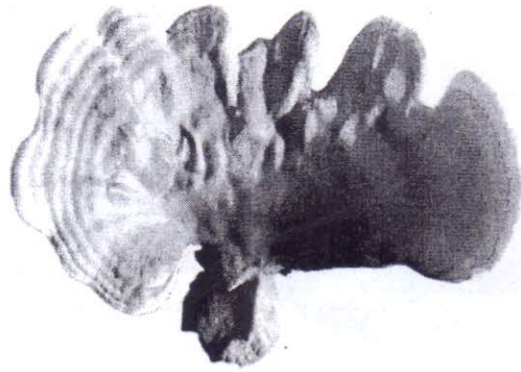
The color, thickness and length of stipes of mature basidiocarps across the isolates exhibited marked variation. The colour of stipe varied from

deep brown in G-1 and G-3 while in G-2 it was brownish. The stipe also appeared laccate in all the specimens. The average thickness of stipe differed from 1.6 cm in G-1 to 2.2 cm in G-2 and 1.4 cm in G-3. The length of stipe varied from 7.5 cm in G-1 to 8.5 cm in G-2 and 10.5 cm in G-3.

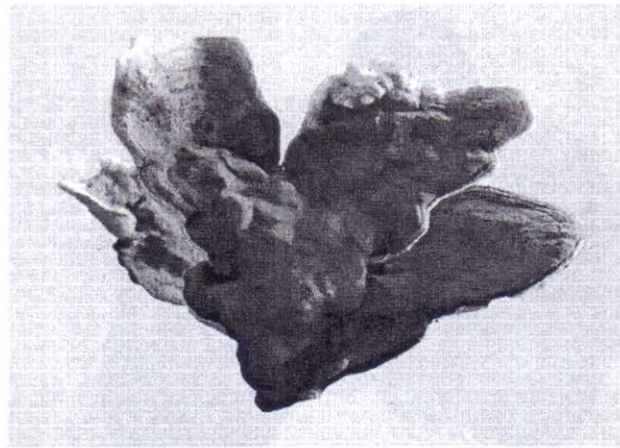
Distinctive morphological characteristics of three *G. lucidum* isolates studied here include colour of upper pilear surface, pilear diameter, colour of context tissue, number of pores per millimetre,



3a : Smooth



3b : Shallow lobed



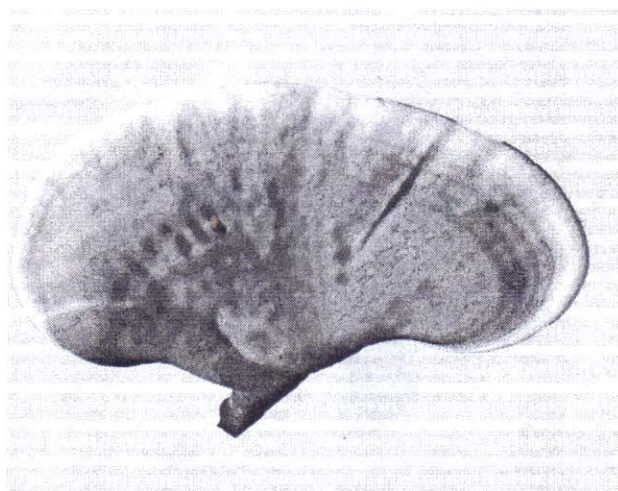
3c : Deep lobed

Fig. 3 : (a-c) Margin patterns of pileus in *Ganoderma lucidum*

shapes of basidiocarps, attachment patterns, margin patterns, surface patterns, stipe colour, stipe thickness and length. The study indicates that variations do occur in these characteristics and the information generated here can find taxonomical application.

The colour variation among isolates has also been observed by Shin and Seo (1988) and thus seems to be a character specific to isolates. The laccate or varnished appearance of the basidiocarp is due to an amorphous substance secreted by the hyphae forming the cover of pilear surface as well as stipe and has been used as an additional feature in classification of *Ganoderma* (Furtado, 1965). The pilear margin which was white in immature and disappeared in mature basidiocarps can

be used as an indicator of maturity. This character also appeared independent of isolates. The colour of context tissue is a useful character in classification and Corner (1983) emphasised the importance of observing the context colour of fresh and living specimens in classification of *Ganoderma*. Number of pores per millimeter may also serve as useful character in classification and may also serve as a specific character (Seo and Kirk, 2000). Shin and Seo (1988) also observed morphological differences among isolates. Morphological variations appear to be affected by environmental conditions during basidiocarp development. Stamets (1993) observed the development of basidiocarp as sensitive to light and ventilation and concluded that under dim light or dark conditions with poor ventilation, abnormal pileus



4a : Smooth



4b : Shallow waved



4c : Deep waved

Fig. 4 : (a-c). Surface patterns of pileus in *Ganoderma lucidum*

shapes of antler type were produced. Both the stipe length and thickness seem to be affected by light and Stamets (1993) reported that stipe exhibited tropic growth towards light.

Basidiocarp characteristics provide information necessary in understanding the species and its better identification. This will help in further clarification of taxonomy of the species which is presently suffering from taxonomic chaos.

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