ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 17(1) 2018, 13–22

www.acta.media.pl ISSN 1644-0692 DOI: 10.24326/asphc.2018.1.2

ORIGINAL PAPER

Accepted: 19.09.2017

WHEAT STRAW – A PROMISSING SUBSTRATE FOR *Ganoderma lucidum* CULTIVATION

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ABSTRACT

Demand for *Ganoderma lucidum* basidiocarps on the global market is increased due to its numerous health benefits. As they are rare in nature and traditional cultivation on logs is not ecologically and economically justified method, current trend is finding of good alternative substrate for production. Whether wheat straw, the most abundant crop residue in Europe, could be a novel substrate for *G. lucidum* cultivation was the question which led to the definition of the goals. Two wild and one commercial strains were objects of the study. Despite some morphological differences among basidiocarps, all strains belong to *G. lucidum sensu stricto*, which was confirmed by analyses of *ITS, tef1-a* and *rpb2* gene sequences. Wheat straw showed as a good substrate, namely the periods required for the complete colonisation of wheat straw by mycelium as well as the formation of primordia and basidiocarps were relatively short. The totally fresh and dry yield and biological efficiency were also significant. If it is taken into consideration that untreated wheat straw was used, validities of its introduction in industrial-scale *G. lucidum* cultivation can be demonstrated.

Key words*:* alternative substrate, basidiocarps, cultivation, *Ganoderma lucidum*, wheat straw

INTRODUCTION

Ganoderma lucidum (W. Curt.:Fr.) P. Karst. is one of the most famous medicinal mushrooms with a long tradition of usage and cultivation, especially in the countries of the Far East. Originally, this species was cultivated on a meter-long unsterilized broad-leaf hardwood logs and a few years of incubation were required for obtaining mature basidiocarps. Currently, *G. lucidum* is a popular dietary supplement, which annual global market value amounts to $1.5 billion [Liu et al. 2010]. That huge demand for its fruiting bodies could be supplied by improvement of traditional cultivation method with the aim of shortening the production period and upgrading fruiting body amount and quality. At the beginning, the improvement was gone in the direction of cultivation on shorter logs [Chen 2002], and later on substrates based on various hardwood sawdusts as well as cereal straws [Mishra and Singh 2006, Erkel 2009a, Gurung et al. 2012, Thakur and Sharma 2015]. Namely, annual world production of crops and other

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lignocellulosic residues is enormous. Despite the fact that they could be a good feedstock for various industries, approximately half of that amount is not used and presents serious environmental ballast [Stajić et al. 2009]. However, owing to well-developed mushrooms 'lignocellulolytic enzymes' systems, those wastes, without any pretreatment, could be good substrates for cheap commercial production of nutritionally high-valued fruiting bodies [Peksen and Yakupoglu 2009]. Nowadays, numerous studies are done with the goal of finding the optimal ecological and economical valid alternative substrates as well as optimization of compost composition for obtaining a high yield of quality fruiting bodies. Namely, effect of various supplements (cereal brans, tea wastes, gluten, molasses etc.), added in different portion to the sawdust/straw based substrates, was tested and significantly higher yield and biological efficiency (even to 27.9%) were noted [Erkel 2009b, Peksen and Yakupoglu 2009, Thakur and Sharma 2015].

Recently, counterfeits of various mushroom based dietary supplements are common. It is specially case with *G. lucidum* products primarily because of fact that it is held to be complex species due to remarkable morphological plasticity among a few related *Ganoderma* species. Nowadays, that problematic taxonomy of the genus is usually overcome by molecular analysis, which can answer what reasons of morphological variations among samples are, whether they are results of point mutations or media/cultivation conditions influences.

Based on the above-mentioned data, the aims of the present study were morpho-physiological and molecular analysis and comparison of autochthonous and commercial *G. lucidum* strains, and testing of wheat straw as a potential alternative substrate for production of basidiocarps. Wheat straw was selected for analysing based on our previous results which demonstrated that commonly substrate for cultivation of *Ganoderma* species, i.e. oak sawdust, was less favourable for synthesis and secretion of ligninolytic enzymes that are responsible for efficient substrate degradation and fungal biomass production [Ćilerdžić et al. 2014, 2016].

MATERIALS AND METHODS

Organisms. Fruiting bodies collected from Bojčin forest near Belgrade (Serbia) from the *Quercus* sp. and Igalo (Montenegro) from the *Platanus* sp. were identified as *Ganoderma lucidum* based on morphological traits (pileus and stipe shape and colour, spores dimensions). Pure cultures were isolated from these basidiocarps as well as commercially cultivated Chinese one, coded as BEOFB 431, BEOFB 434 and BEOFB 432, respectively, maintained on Malt agar medium (pH 6.5) at a temperature of 4°C in the culture collection of the Institute of Botany, Faculty of Biology, University of Belgrade (BEOFB), and re-inoculated every month.

DNA isolation and sequence analysis. Genomic DNA was extracted from dried fruiting bodies using a Dneasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Specific primer pairs ITS5/ITS4 were used for PCR amplifications of the internal transcribed spacer region ITS1-5.8S-ITS2 of the ribosomal genes. The *tef1-a* and *rpb2* gene fragments were amplified using the primer pairs EF1-983F/EF1-2218R, and fRPB2-5F/bRPB2-7R2, respectively.

The PCR procedure was composed of initial denaturation (5 min at 94°C), 35 cycles of 30 s at 94°C, annealing temperatures for 30 s and 90 s at 72°C, and a final extension step (10 min at 72°C). The annealing temperatures for various primer pairs were as follows: 52°C (ITS5/ITS4), 60°C (EF1-983F/EF1-2218R) and 55°C (fRPB2-5F/bRPB2-7R2). Amplification reactions were performed in a Mastercycler personal model (Eppendorf, Hamburg, Germany) in a 25 μL reaction mixture using the following final concentrations or total amounts: 30.0 ng of genomic DNA, 1 × PCR buffer (20.0 mMTris/HCl pH 8.4, 50.0 mMKCl), 1.0 μM of each primer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 1.0 unit of Taq polymerase (Fermentas, Vilnus, Lithuania). The PCR amplified DNA fragments were fractionated on 1% agarose gels in 0.5 × TBE buffer and visualised by ethidium bromide staining and UV illumination. The resulting PCR products were separated by electrophoresis and the bands were purified using

a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) for sequencing (Macrogene, Seoul, Korea). Sequences were compared with other related sequences of *Ganoderma* representatives using BLAST program of NCBI for primary identification and better understanding taxonomical position and phylogenetic relationship (tab. 1). Fungal sequences were deposited in NCBI GenBank under the accession numbers presented in Table 1.

ITS, tef1-a and *rpb2* partial gene sequences were obtained to determine preliminary identification at higher taxonomy level using alignment tool search BLAST. Manual corrections of the aligned database, multiple alignments, and comparisons among strains were conducted using MEGA 6 software package. These gene sequences were assembled and edited using FINCHTV v.1.4.0 (http://www.geospiza.com). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the analysed taxa. The evolutionary distances were computed using the Maximum Composite Likelihood method [Tamura et al. 2004] and presented in the units of the number of base substitutions per site.

Table 1. Information on species used in phylogenetic analysis

Specie/Isolates	Reference	GenBank Accession numbers		
		ITS	*tef1-a*	*rpb2*
Ganoderma boninense WD 2028 (FFPRI)	Zhou et al. 2015	KJ143905	KJ143924	KJ143964
Ganoderma boninense WD 2085 (FFPRI)	Zhou et al. 2015	KJ143906	KJ143925	KJ143965
Ganoderma curtisii CBS 100131	Zhou et al. 2015	JQ781848	KJ143926	KJ143966
Ganoderma curtisii CBS 100132	Zhou et al. 2015	JQ781849	KJ143927	KJ143967
Ganoderma lucidum Cui 9207 (BJFC)	Zhou et al. 2015	KJ143910	KJ143928	KJ143970
Ganoderma lucidum K 175217	Zhou et al. 2015	KJ143911	KJ143929	KJ143971
Ganoderma lucidum MT 26/10 (BRNM)	Zhou et al. 2015	KJ143912	KJ143930	–
Ganoderma multipileum CWN 04670 (TNM)	Zhou et al. 2015	KJ143913	KJ143931	KJ143972
Ganoderma multipileum Dai 9447 (IFP)	Zhou et al. 2015	KJ143914	KJ143932	KJ143973
Ganoderma oregonense CBS 265.88	Zhou et al. 2015	JQ781875	KJ143933	KJ143974
Ganoderma tsugae Dai 12751b (BJFC)	Zhou et al. 2015	KJ143919	KJ143960	KJ143977
Ganoderma tsugae Dai 12760 (BJFC)	Zhou et al. 2015	KJ143920	KJ143940	KJ143978
Ganoderma zonatum FL-02 (TNM)	Zhou et al. 2015	KJ143921	KJ14394	KJ143979
Ganoderma lucidum HMAS 86597	Wang et al. 2012	AY884176	–	JF915436
Ganoderma lucidum G1T099	Wang et al. 2012	AM269773	–	–
Ganoderma resinaceum CBS 194.76	Zhou et al. 2015	KJ143916	KJ143934	–
Ganoderma resinaceum Rivoire 4150	Zhou et al. 2015	KJ143915	–	–
Ganoderma lucidum Rivoire 4195	Zhou et al. 2015	KJ143909	–	KJ143969
Ganoderma sessile JV 1209/27	Zhou et al. 2015	KF605630	KJ143937	KJ143976
Ganoderma lucidum CGMCC 5.75	Wang et al. 2012	JN197282	–	JN197288
Ganoderma resinaceum Boud. HMAS 86599*	Wang et al. 2012	AY884177	–	JF915435
Ganoderma resinaceum Boud. GrTO 96	Wang et al. 2012	AM906065	–	–
Ganoderma lucidum BEOFB 431	This study	KX371594	KX371597	KX371600
Ganoderma lucidum BEOFB 432	This study	KX371595	KX371598	KX371601
Ganoderma lucidum BEOFB 434	This study	KX371596	KX371599	KX371602

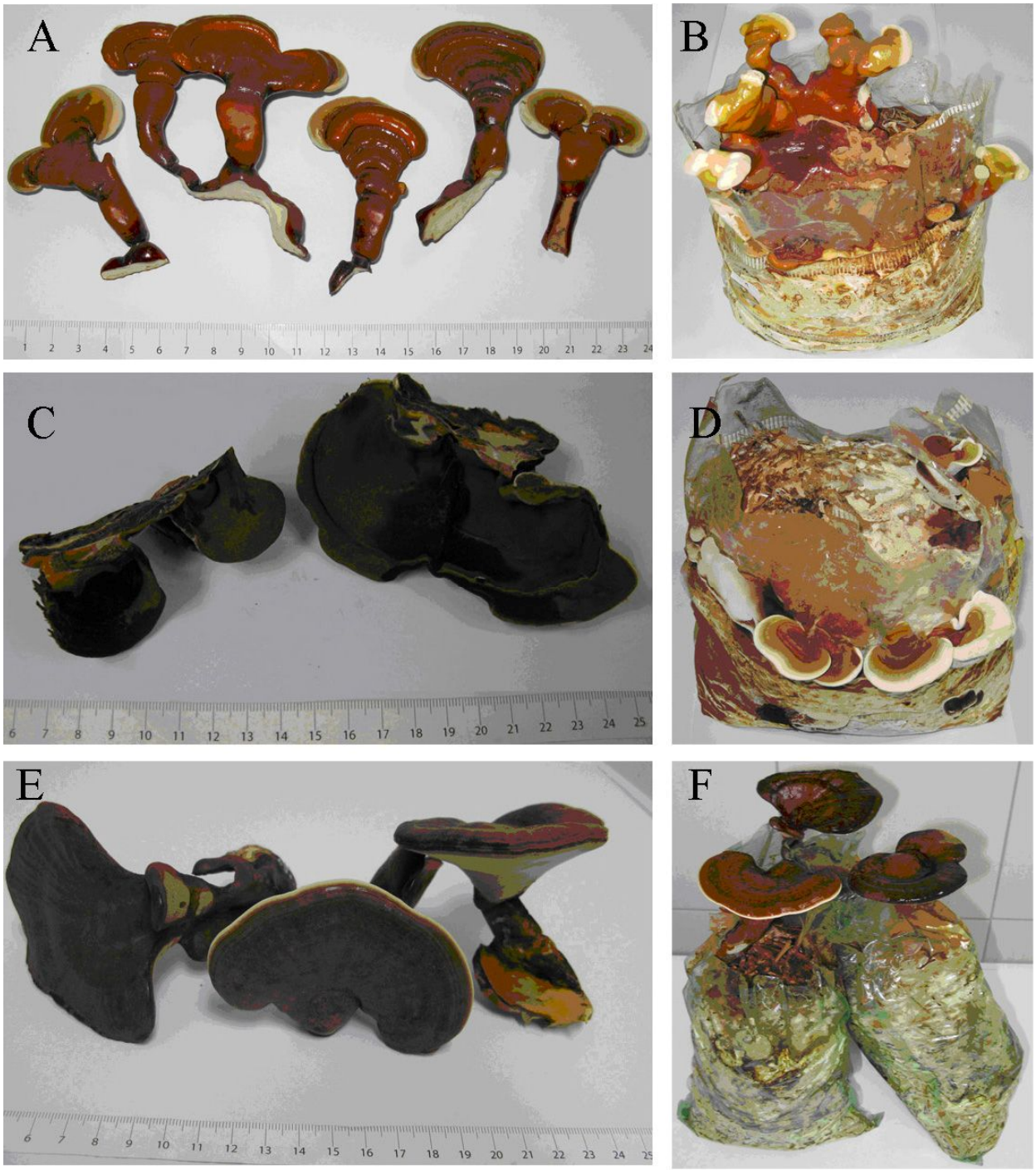

Fig. 1. *Ganoderma lucidum* basidiocarps obtained in cultivation on wheat straw: A, B – *G. lucidum* BEOFB 431; C, D – *G. lucidum* BEOFB 432; E, F – *G. lucidum* BEOFB 434

Mushroom cultivation. Spawn preparation consisted of several steps: (i) washing and soaking of wheat grains in fresh water for 30 min, (ii) boiling of wheat grains in equal volume of fresh water and imbibing till the grains become soft (for 45 min), (iii) addition of $CaCO_3$ and $CaSO_4 \times H_2O$ to the boiled wheat grains in order to adjust the optimum pH value (pH 7.0), (iv) filling of glass jars with prepared grains and sterilization at 121°C for 2 h, (v) inoculation with square cuts of 7-days old culture, and (vi) incubation in the dark at 25°C for 10 days.

The wheat straw, without any additives was used as a substrate for basidiocarps cultivation. Substrate preparation was performed in several stages: (i) fragmentation of wheat straw by secateurs in particles of about 2.0 cm and soaking them in boiling water overnight, (ii) filling polypropylene filter bags ($SacO_2$) with the tightly compacted wheat straw (1000 g), which humidity was about 62%, and plugging them with cotton plugs, (iii) sterilization at 121°C for 2 h, (iv) cooling the substrate at the room temperature in the sterile chamber overnight, and (v) inoculation with 10 g of prepared spawn (1% of substrate amount).

Substrate colonisation by mycelium was performed by incubation at 25°C in the dark during 15 to 20 days. After complete substrate ingrowth, the bags were transferred to growth chamber (Lab Companion GC-1000TLH, Seoul, Korea), the cotton plugs were removed and a process of fructification has been started. The cultivation conditions were adjusted according to Stamets (1993): day-night light regime (12 hours on/off, 1000 lux); temperature of 18 to 20°C; relative air humidity of 90%; CO_2 concentration less than 2000 ppm (fresh air exchanges were performed as required for maintaining desired CO_2 concentration).

The time required for the complete substrate colonisation by mycelium, as well as that from the bag opening to the primordia and fruiting bodies formation was measured. A number of fruiting bodies per bag (1000 g of initial substrate) and some morphological characteristics (diameter of pileus and stipes, a length of stipes, and a number of generational zones) were observed. The total fresh and dry yield (g kg^{-1} of the substrate) were recorded by weighing the mass of obtained fruiting bodies before and after the drying process at 60°C. Biological efficiency was determined according to the formula:

$$\text{Biological efficiency (\%)} = [\text{Total fresh yield (g)} / \text{Total dry substrate (g)}] \times 100$$

Statistical analysis. The assays were carried out in triplicate and the results are expressed as a mean ± standard error. One-way analysis of variance (ANOVA) and Tukey's test were performed using STATISTICA, version 6.0 (StatSoft, Inc., Tulsa, USA) to test any significant differences among means. Statistical significance was declared at $P < 0.05$.

RESULTS

Morphological and molecular features of the studied samples. The fruiting bodies of studied *Ganoderma lucidum* strains showed the significant macromorphological differences (fig. 1 C, D). The pileus surface of the strain BEOFB 432 was mat and dark brown, slightly concave in the middle, with 3 to 5 generational zones and diameter ranged from 3.5 to 11.0 cm, and stipes was absent (tab. 2). Contrary to those sessile and brown basidiocarps, wild ones had stipes and pileus of vivid colours. Thus, basidiocarps collected from *Platanus* sp. on Montenegro seaside (BEOFB 434) had a shiny red-brown surface, pileus diameter of 6.0 to 13.0 cm, and stipes length in the range from 5.5 to 8.0 cm (fig. 1 E, F; tab. 2). The radiant orange-brown surface of pileus whose diameter was ranged from 3.0 to 8.2 cm and stipes length from 5.5 to 8.0 cm were the characteristics of the fruiting bodies originated from *Quercus* sp. from Belgrade suburb (BEOFB 431) (fig. 1 A, B; tab. 2).

These noted variations led to question whether studied samples present various strains of the same species or belong to different species of the *G. lucidum* complex. Molecular analysis, namely comparison of ITS, *tef1-a* and *rpb* sequences of studied samples with the same ones of related *Ganoderma* spp. deposited in NCBI GenBank (tab. 1), gave a response

to the question. Based on ITSregion sequences, studied isolates were 99% homologous with *Ganoderma resinaceum* voucher HMAS86599 from GenBank (accession number KT906371) and 98% with *G. lucidum* ATCC 64251 (accession number JQ520187). Usage of the obtained data and Maximum Composite Likelihood method phylogenetic tree was constructed, and it can be observed that studied samples are phylogenetically very close and present *G. lucidum sensu stricto* (fig. 2).

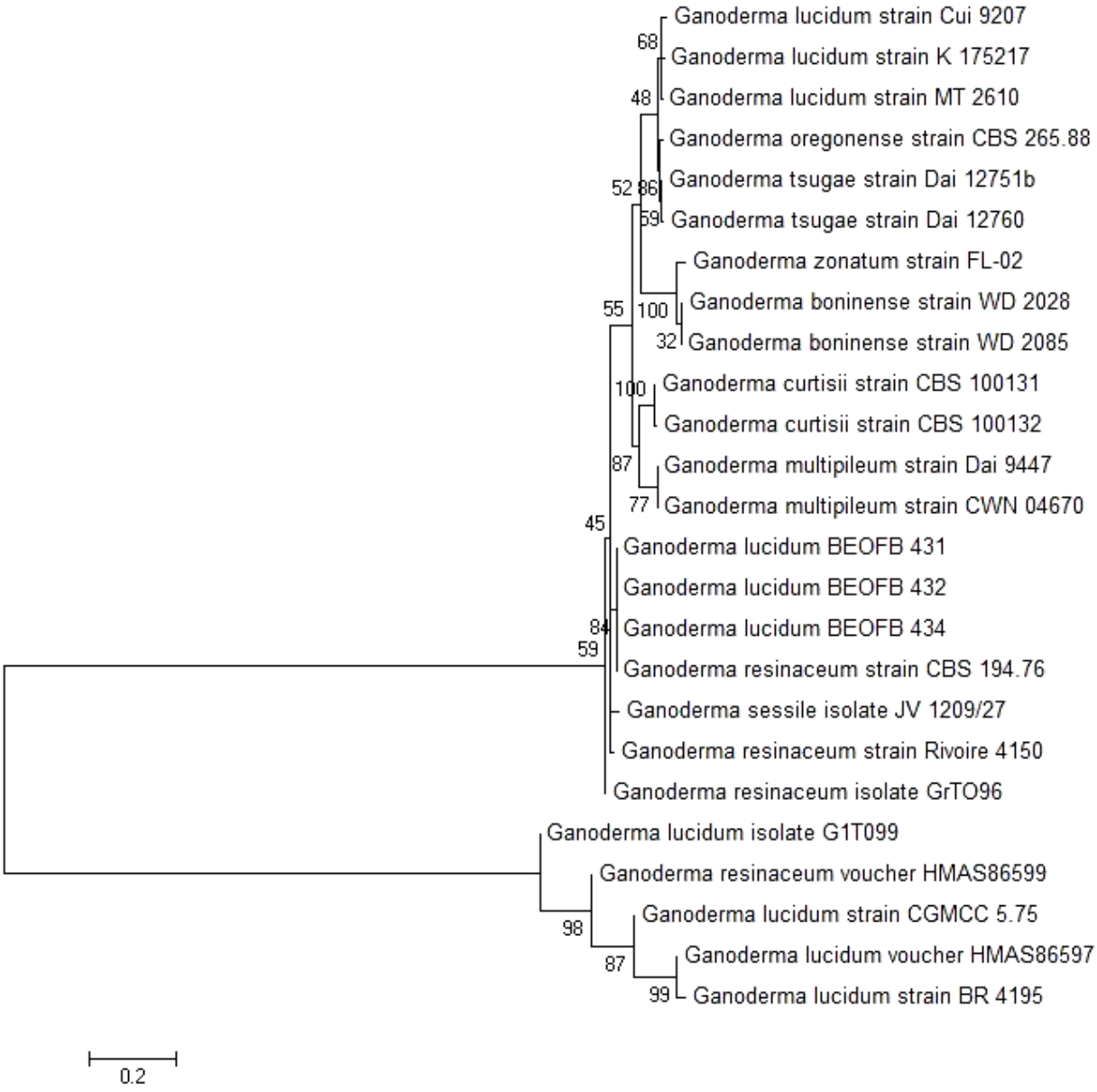

Fig. 2. Maximum Likelihood tree based on combined data set of ITS, *tef1-a* and *rpb2* partial gene sequences

Table 2. Morpho-physiological characteristics of *Ganoderma lucidum* basidiocarps cultivated on wheat straw

Studied characteristics	*Ganoderma lucidum* strains		
	BEOFB 431	BEOFB 432	BEOFB 434
Period of substrate colonization by mycelium (day)	17.7 ±1.2[a*]	17.3 ±1.4[a]	18.0 ±1.2[a]
Period required for primordia formation (day)	22.0 ±1.8[a]	21.0 ±2.1[a]	25.7 ±2.0[b]
Period required for basidiocarp formation (day)	49.0 ±1.4[b]	46.7 ±1.4[a]	54.7 ±1.7[c]
Number of basidiocarps (per bag)	8–12[b]	8–13[b]	1[a]
Pileus diameter (cm)	3.0–8.2[a]	3.5–11.0[b]	6.0–13.0[c]
Stipes length (cm)	3.5–8.0[a]	/	5.5–8.0[b]
Stipes diameter (cm)	0.7–3.5[a]	/	1.2–3.0[b]
Number of generational zones (rings)	1–6[a]	3–5[b]	3–5[b]
Total fresh yield (g kg^{-1} of substrate)	43.8 ±2.8[b]	40.8 ±3.6[b]	20.8 ±2.0[a]
Total dry yield (g kg^{-1} of substrate)	14.0 ±0.9[b]	13.1 ±1.1[b]	6.7 ±0.7[a]
Biological efficiency (%)	13.0 ±0.9[b]	12.0 ±1.2[b]	6.0 ±0.9[a]

*Mean ± standard error. Values within a row with different superscripts differ significantly ($p < 0.05$) according to Tukey's test

Validity of wheat straw usage for *Ganoderma lucidum* cultivation. Wheat straw was shown as a good alternative substrate for *G. lucidum* cultivation due to a few reasons. The first one, time period required for complete colonisation of wheat straw by mycelium was relatively short, from 17.3 days in *G. lucidum* BEOFB 431 to 18.0 days in *G. lucidum* BEOFB 434. No statistically significant differences among the studied strains in the mycelial growth rate were noted. Secondly, primordia were formed only after 3.7 (BEOFB 432), 4.3 (BEOFB 431), and 5.7 days (BEOFB 434) after opening the cultivation bags, and fruiting bodies from 25.7 days (BEOFB 432) to 29.0 days (BEOFB 434) after that. Basidiocarps' number per bag as well as their total fresh and dry yields were considerable especially in strain BEOFB 431 (8–12 basidiocarps, 43.8 g kg^{-1} and 14.0 g kg^{-1} of the substrate, respectively), which was also characterised by biological efficiency of 13% (tab. 2).

DISCUSSION

Initially, the genus *Ganoderma* was classified based on morphological features into two groups, laccate *G. lucidum* species complex and non-laccate *G. applanatum* species complex [Hapuarachchi et al. 2015]. However, as morphology is variable and depends on environmental factors and as inter-hybridization within a genus is the common phenomenon, identification of species, especially into the *G. lucidum* complex, becomes the serious problem. Therefore, Richter et al. [2015] suggested that taxonomy of the genus should be based on the combination of morphological, chemical, and molecular criteria. Thus, Moncalvo et al. [1995a, b] and Hong and Jung [2004] showed by analysis of ITS region as well as sequences of partial large subunit rDNA, mitochondrial small subunit rDNA and partial β-tubulin genes, that significant morphological differences among European/Canadian and East Asian *G. lucidum* strains, reported by Pegler and Yao [1996], have molecular base. Based on those molecular data, Asian *G. lucidum* was clustered into the separated group, and Hawksworth [2005] and Wang et al. [2012] even introduced new names. Contrary to numerous records [Moncalvo et al. 1995a, Mohanty et al. 2011, Zhou et al. 2015] according which *G. lucidum* and *G. resinaceum* (synonym *G. sessile*) are significantly different,

Hapuarachchi et al. [2015] emphasized their closely relation and placed them in the same clade. According to everything mentioned, molecular analysis of all three strains tested in our study showed that they belong to the same clade and confirmed their identification based on morphological traits as *G. lucidum sensu stricto*.

This study presents a great contribution to improvement and discount of *G. lucidum* cultivation because the significant yield was obtained by growing on untreated wheat straw for short period. The results obtained for *G. lucidum* BEOFB 431 were better than those obtained in *G. lucidum* cultivation on substrate composed of mixture of eight different sawdust kinds (*Mangifera indica*, *Eucalyptus camaldulensis*, *Tectona grandis*, *Albizia richardiana*, *Bombax ceiba*, *Albiziaprocera*, and *Borasus flabellifer*) where the obtained total fresh and dry yields were 22.0 and 6.13 g per 500 g of substrate, respectively, and biological efficiency was 11% [Hossain et al. 2009]. Likewise, complete colonisation of *Eucalyptus camaldulensis* sawdust and logs lasted significantly longer than in the case of wheat straw (26.25 days and 60 days, respectively) [Chen 2002, Hossain et al. 2009, Gurung et al. 2012, Kamra and Bhatt 2013]. Numerous studies also showed that wheat straw was more appropriate substrate for mycelium running, as well as primordia and fruiting bodies development in other mushroom species [Philippoussis et al. 2007, Chae and Ahn 2013, Postemsky and Curvetto 2015]. Thus, Philippoussis et al. [2007] reported that fruiting bodies of *Lentinula edodes* appeared earlier on wheat straw than on oak sawdust (48 days *vs*. 60 days) and that biological efficiency and basidiocarps' number per bag were about twice higher in cultivation on straw than on sawdust (75% and 41%, respectively, and 25 and 12 fruiting bodies per bag, respectively). Gurung et al. [2012] reported that sawdust and logs of *Alnus nepalensis*, *Shorea robusta*, and *Dalbergia sisoo*, alone or enriched with different cereals brans, were also worse substrates in comparison with wheat straw, as the highest noted total yield (37.62 g kg^{-1} of *A. nepalensis* sawdust) was lower by 14% than that in *G. lucidum* BEOFB 431 cultivated on wheat straw. Testing various lignocellulosic residues, Chae and Ahn [2013] revealed sawdust as a less favourable substrate for *Pleurotus ostreatus* fructification, while Postemsky and Curvetto [2015] observed that wheat straw induced significant colonisation rate in *Grifola gargal*. These results could be explained by the production of more active ligninolytic enzymes isoforms during *Ganoderma* spp. cultivation on wheat straw. Ćilerdžić et al. [2014, 2016] reported that Mn-oxidizing peroxidases and laccases were about 10- and 2.5-fold more active, respectively, in cultivation on wheat straw than on oak sawdust.

However, contrary to those data, Erkel [2009a] reported that oak, poplar and beech sawdusts enriched with wheat, rice or corn bran, were more efficient substrates than wheat straw. The total obtained yield was in the range from 47.72 g kg^{-1} of poplar to 60.24 g kg^{-1} of oak sawdust, and biological efficiency was from 15% (on poplar sawdust) to 17% (on oak sawdust). Thakur and Sharma [2015] also noted higher yield and biological efficiency on substrates based on various sawdusts enriched with wheat bran. Further enrichment of sawdust/bran substrates with additional carbon and nitrogen sources led to further increase of total yield and biological efficiency. Thus, Erkel [2009b] obtained production higher in 36% to 40% and efficiency of 19% to 20% after enrichment of poplar sawdust/wheat bran compost with 1% molasses and 1% corn gluten. Significant *G. lucidum* production (99.88 g kg^{-1} of the substrate) and biological efficiency (40%) were also noted by Peksen and Yakupoglu [2009] who used substrate composed of *Carpinus betulinus* sawdust and tea-waste where C/N ratio was 80% : 20%. The great potential of sawdust enriched by various bran and flour for *G. lucidum* basidiocarp production was also demonstrated by Kamra and Bhatt [2013] who noted a total yield of even 190.0 g kg^{-1} of the substrate. However, Royse and Bahler [1986] emphasised that the biological efficiency was significantly affected not only by substrate composition, but also by genotype and spawn running period that depends on substrate C/N ratio, aeration level, and water holding capacity. Thus, C/N ratio of 70 in the substrate composed of soy residue and *Acacia confusa* sawdust was the

optimal ratio for its fast colonisation in only 16 days, while with a decrease of the ratio colonisation period extended [Hsieh and Yang 2004].

Numerous studies have also demonstrated that period required for *G. lucidum* primordia and basidiocarp formation was different depending on substrate type and composition. Thus, comparing with our study, longer periods (40 to 86 days) were needed for primordia and for basidiocarp formation (60 to 92 days) on various broad-leaf tree sawdusts [Gurung et al. 2012, Kamra and Bhatt 2013] and even 110 to 120 and 135 to 145 days, respectively, on the logs [Chen 2002]. The C/N ratio in the substrate had also a significant effect on these periods [Hsieh and Yang 2004]. Namely, these authors obtained fruiting bodies only in C/N ratio of 80 and 70, after 48 and 49 days of cultivation, respectively.

The substrate is also responsible for shape, dimension, and a number of produced basidiocarps. That was demonstrated by Yang et al. [2003] who obtained from two to even 10 basidiocarps per bag, and depending on the number, basidiocarps were without or with antler branches and had dimensions from 4.1 × 3.2 cm to even 11.0 × 7.5 cm. According to this criteria, wheat straw as a new substrate and autochtonous *G. lucidum* strains (BEOFB 431 and BEOFB 432) were better, namely, more of larger fruiting bodies were produced per bag. Hossain et al. [2009] confirmed that the kind of sawdust significantly affects basidiocarp dimension but they have not found any differences in their number. Thus, stipes length was the highest in basidiocarps obtained on *Albizia procera* sawdust (2.68 cm) contrary to those on *Mangifera indica* sawdust (1.18 cm), while pileus diameter was the highest on substrates composed of sawdust mixture (6.05 cm) and the smallest on *Borasus flabellifer* sawdust (4.0 cm), which was significantly lower than in fruiting bodies of *G. lucidum* BEOFB 431 and BEOFB 434 cultivated on wheat straw.

CONCLUSION

This study clearly showed that the shorter time period is required for *Ganoderma lucidum* mycelium running as well as primordia and fruiting bodies formation on wheat straw than on other commonly used substrates. High values of total yield and biological efficiency were obtained during cultivation on wheat straw, without any pretreatment and enrichment, which recommends it as a good alternative substrate for *G. lucidum* cultivation in industrial-scale. This is very important especially considering the fact that wheat straw is one of the most abundant and accessible crop residues so *G. lucidum* basidiocarps could be produced cheaper than by usage of the traditional method.

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