

Breeding and Evaluation of *Pleurotus giganteus* (Berk.) Karunarathna & K.D. Hyde Hybrids via Intraspecific Mating

(Pembiakbakaan dan Penilaian Hibrid *Pleurotus giganteus* (Berk.) Karunarathna & K.D. Hyde melalui Kacukan Intraspesies)

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ABSTRACT

Pleurotus giganteus, the giant oyster mushroom is gaining popularity as a gourmet mushroom. The cultivation of this mushroom in Malaysia, however, is facing challenges such as malformed pinhead formation and low yield resulting in heavy economic losses for farmers. The aim of this study was to generate new hybrids of *P. giganteus* with higher yield for commercialisation in Malaysia. The commercial strain of *P. giganteus* (China) (KLU-M1227) and a wild strain from Malaysia (KLU-M1391) were subjected to intraspecific breeding. A total of 16 hybrids were obtained from 56 combinations. All the parents and hybrids were subjected to linear growth assessment. The hybrids were clustered into slow-growing (growth rate < 0.29 cm/day) and fast-growing (growth rate in 0.30 cm/day) types. The parents and three hybrids (IH15, IH17 and IH32) that were the least susceptible to *Trichoderma* sp. were then cultivated using soil-casing method. This method was able to solve long, coiling stipe formation of *P. giganteus*. The parent and hybrid strains showed variation in the number of days for pinhead formation and number of fruiting bodies formed. The hybrid with the highest yield (IH32) had a 32% higher biological efficiency compared to both the parents. Inter simple sequence repeats (ISSR) amplifications were carried out to confirm the generation of the hybrids. Hybrids IH17 and IH32 contained the genetic materials inherited from both parents while hybrid IH15 shared high amount of genetic materials from one parent.

Keywords: Biological efficiency; giant oyster mushroom; hybrid; linear mycelial growth

ABSTRAK

Pleurotus giganteus yang lebih dikenali sebagai cendawan tiram gergasi dalam kalangan masyarakat, kini semakin popular sebagai salah satu cendawan gourmet. Walau bagaimanapun, penanaman cendawan ini di Malaysia sedang mengalami pelbagai masalah seperti kecacatan pembentukan putik dan hasil yang rendah lalu menyebabkan kerugian besar kepada petani. Tujuan utama kajian ini adalah untuk menjana hibrid baru *P. giganteus* dengan hasil yang tinggi untuk dikomersilkan di Malaysia. Pembiakbakaan intraspesies telah dijalankan bagi strain komersil *P. giganteus* (China) (KLU-M1227) dan strain liar dari Malaysia (KLU-M1391). Sebanyak 16 hibrid berjaya diperolehi daripada 56 gabungan kacukan. Penilaian pertumbuhan linear telah dilakukan bagi induk dan hibrid. Hibrid telah dikelompokkan kepada jenis pertumbuhan perlahan (kadar pertumbuhan < 0.29 cm/hari) dan pertumbuhan pantas (kadar pertumbuhan \geq 0.30 cm/hari). Induk dan tiga hibrid (IH15, IH17 dan IH32) yang paling kurang kerentanan terhadap *Trichoderma* sp. dipilih untuk ditanam menggunakan kaedah salutan tanah. Kaedah ini mampu untuk menyelesaikan masalah pembentukan tangkai buah *P. giganteus* yang panjang dan berlingkar. Strain induk dan hibrid menunjukkan variasi dalam jumlah bilangan hari bagi pembentukan putik cendawan dan pembentukan badan berbuah. Hibrid dengan hasil tertinggi (IH32) mempunyai kecekapan biologi 32% lebih tinggi berbanding kedua-dua induk. Teknik amplifikasi Inter Simple Sequence Repeats (ISSR) telah dijalankan untuk menentukan generasi hibrid. Hibrid IH17 dan IH32 didapati mengandungi bahan genetik yang diwarisi daripada kedua-dua induk manakala hibrid IH15 mewarisi bahan genetik yang banyak daripada satu induk.

Kata kunci: Cendawan tiram gergasi; hibrid; kecekapan biologi; pertumbuhan linear misel

INTRODUCTION

In recent years, commercial mushroom production has increased worldwide contributing to high economic returns to exporting countries such as Poland, China

and the United States (Wakchaure 2011). According to the National Agro-Food Policy 2011-2020 (MOA 2019), the Ministry of Agriculture (MOA) of Malaysia regards mushroom as an important food crop which has the potential to contribute to food security and economic

gains for the country. In Malaysia, *Pleurotus* species such as *P. pulmonarius*, *P. floridanus*, and *P. cystidiosus* are in demand by the Malaysian consumers. The culinary giant oyster mushroom, *P. giganteus*, though new to Malaysians, is gaining popularity due to its chunky texture and seafood flavour (Nas Agro 2013 personal communication).

P. giganteus has high contents of magnesium, potassium, amino acids, iron and calcium which may benefit human health (Phan et al. 2019, 2014). Furthermore, the medicinal properties of *P. giganteus* that have been reported are hepatoprotective (Wong et al. 2012), stimulation of neurite outgrowth (Phan et al. 2012), reduction of isolated skeletal muscular fatigue (Dong et al. 2010; Huang et al. 2005), and inhibition of growth of *Candida* species (Phan et al. 2013).

P. giganteus, previously reported as *Lentinus giganteus* or *Panus giganteus*, consists of large fruiting body and is easily recognised (Karunarathna et al. 2011). In Malaysia, it is known as 'cendawan seri pagi' (morning glory mushroom). Wild *P. giganteus* is widely consumed but still not commercialised in Sri Lanka and Thailand (Klomklung et al. 2012). Apart from these countries, occurrence of wild *P. giganteus* has been reported in Vietnam, Australia (Pegler 1983), China (Bi et al. 1993; Phan et al. 2012) and Oceania (Bi et al. 1993).

P. giganteus is one of the popular mushrooms in China, and its biological characteristics, nutritional value and cultivation methods had been studied since 1990 (Dong et al. 2010). Karunarathna et al. (2011) reported that cultivation of *P. giganteus* in Thailand may introduce a high income earning mushroom industry. Klomklung et al. (2012) reported that this mushroom took a long time to fruiting and the yield was low, which may contribute to a higher market price compared to other mushroom species.

In Malaysia, the wild *P. giganteus* has been found growing on the soil in villages in Negeri Sembilan (Batin Long, personal communication, Apr 4, 2014; Batin Seturi, personal communication, Jul 2, 2013). *P. giganteus* has not been widely commercialised in Malaysia. It was once cultivated at NAS Agro Farm, Sepang (Mr. Nabil, personal communication, Apr 2, 2013). However, the cultivation was discontinued mainly due to severe contamination by fungi. This species was reported to form long coiling stipes in the substrate bag. After harvest, the stipes which remained inside the mushroom bags attracted insects, leading to fungal contamination, infestation with maggots and loss in yield. Sonnenberg et al. (2005) suggested a breeding hybridisation strategy using wild and commercial strains as parents might result in hybrids with beneficial commercial traits. Furthermore, Kothe (2001) emphasised the importance of mating studies and breeding programs to generate improved strains with greater resistance to fungal

contamination and higher yield.

Inter simple sequence repeats (ISSR) marker approach took advantage of simple sequence repeats (SSR) or microsatellites that are common throughout the eukaryotic genome by amplifying the region between two similar microsatellites. The method is reproducible and relatively low in cost, time and labour. ISSR methodology is widely applied in several fields of plant research including genomic fingerprinting, genetic diversity, phylogenetic analysis, genome mapping, gene tagging, marker assisted selection, SSR motif frequency determination and evolutionary biology (Reddy et al. 2002). For macrofungi, ISSR markers have been used in the study of genetic diversity (Yin et al. 2014; Zhang et al. 2012), genotyping (Urbanelli et al. 2007) and determining phylogenetic relationships (Sahoo et al. 2014). There is a minimal usage of ISSR markers for identification of hybrids in mushroom related research (Doroteo et al. 2018), while such studies are very common in plant breeding programs (Bianco et al. 2011; Gradzielewska et al. 2012).

In this study, the strains studied were a commercial strain from China collected by NAS Agro Farm and a wild strain as parents. The aims of this study were to evaluate the growth performance and cultivation technique of hybrids produced, and verify the selected hybrids using ISSR markers.

MATERIALS AND METHODS

FUNGAL STRAINS

The commercial strain *Pleurotus giganteus* KLU-M1227 was obtained from NAS Agro Farm, Selangor, Malaysia. Meanwhile, the wild strain KLU-M1391 was found caespitose on soil within the vicinity of University of Malaya, Kuala Lumpur. Both strains were identified as *P. giganteus* by Mushroom Research Centre, University of Malaya based on macro-morphological characteristics and molecular analysis. All the fungal strains were maintained as stock cultures in the laboratory.

MATING STUDIES

ISOLATION OF SINGLE SPORE CULTURES

Monokaryons were obtained from a small piece of mushroom lamella by the spore drop method (Petersen & Bermudes 1992). The lamella was attached to the cover of potato dextrose agar (PDA) (Difco) plate using vaseline. The agar plate was sealed and incubated at room temperature (26 ± 2 °C) at a slanting angle for 24 h. The lamella was then removed. The agar plate was incubated at room temperature until the basidiospores germinated. The plate was observed under dissecting microscope for germinating single spores. With the help of a modified fine inoculation needle, germinated single

spores were transferred onto new PDA. The monokaryons on PDA were kept as stock cultures in sterile distilled water and refrigerated at 4 °C (Castellani 1968).

MATING OF MONOKARYONS FROM DIFFERENT STRAINS

Fast-growing monokaryon cultures of the two parents were selected for intraspecific mating. Agar plugs of each isolate were placed on PDA plates, 10 mm apart from each other. The plates were then incubated at room temperature until the mycelia made contact. The mycelium was picked from the contact zone using a needle, mounted on a slide and then checked under compound microscope for clamp connection. The contact zone of the isolates was then cut with a sterile blade and transferred onto a new PDA plate (Bao et al. 2004). Hybrids were kept as stock cultures in sterile distilled water and refrigerated at 4 °C (Castellani 1968).

LINEAR GROWTH STUDY IN A RACE TUBE

The substrate consisting of 89% (w/w) sawdust, 10% (w/w) rice bran and 1% (w/w) calcium carbonate was prepared in glass race tubes (160 mm × 25 mm). The moisture content of the substrate was 73 to 74%. The substrate (58.50 g) was compressed to 100 mm in length. The tube was stoppered with non-absorbent cotton and then sterilised at 121 °C for 15 min. A mycelial disc (1 cm) was taken from a 10 days old culture and then transferred to the substrate surface. The tubes were incubated at 26±2 °C in the dark. Visible mycelial extension was recorded at two perpendicular directions every 3 days up to day 15. The substrate inoculated with the parents was used as control. This experiment was carried out in triplicates for each parent and 16 hybrid cultures generated from mating of monokaryons from different strains.

CULTIVATION OF THE PARENTS AND SELECTED HYBRIDS

Both the parents and selected hybrids of *P. giganteus* namely IH15, IH17, and IH32 which were the only fast growing strains with dense mycelium and susceptible to pathogen testing (unpublished data) selected from linear growth study were cultivated on sawdust substrate using the conventional method practiced in mushroom farms with and without soil-casing. Mushroom bags consisting of 73 to 74% (v/w) water, 89% (w/w) sawdust, 10% (w/w) rice bran and 1% (w/w) calcium carbonate were prepared. The substrate (300 g) was packed tightly and a central hole was made with the help of a 50 mL centrifuge tube. The bags were then stoppered with caps and sterilised at 121 °C for 15 min. After sterilisation, the bags were allowed to cool to room temperature for 24 h. The bags were then inoculated with spawn prepared on wheat grains and were then incubated in the dark at 26±2 °C. When the bag was 50% colonised by the mycelium, the wheat grains were removed from the substrate with a sterile spatula in order to reduce contamination by

fungi. In the conventional method, 1 week after the mycelia had completely colonised the substrate, the bags were transferred to the mushroom house at Institute of Biological Sciences, Faculty of Science, University of Malaya. The relative humidity around 70% and a temperature of 29±6 °C was maintained in the house. The caps of the mushroom bags were removed for fruiting to occur. For the soil-casing method, garden soil mixed with coconut husks was bought from a nursery at Sungai Buloh, Selangor and sterilised at 121 °C for 15 min. The soil was slightly moistened with tap water and then 2 cm height of soil was layered on top of the substrate. The plastic covering of the bag was folded down until it was approximately 3 cm above the soil layer. Three replicates were set up for each parent and selected hybrids for each cultivation method. The fruiting bodies before maturity (the edge of the pileus was wavy) were harvested by hand. Data such as the time required for spawn completion, duration from stimulation to pinhead emergence, duration from stimulation to harvesting, total weight of fruiting bodies, pileus diameter, stipe length and stipe thickness were recorded (Klomklung et al. 2012). Color descriptions of the fruiting bodies were recorded by referring to Kornerup and Wanscher (1963). The biological efficiency was calculated as given by the formula below:

$$\text{Biological efficiency} = \frac{\text{Total weight of fresh fruit bodies}}{\text{Dry weight of substrate}} \times 100$$

Cultivation method which gave a higher biological efficiency results in all the soil-casing method was then adopted to test the parents and selected hybrids. After harvesting, tissues were transferred from the fruiting bodies of the parents and selected hybrids to PDA using a sterile forceps. Mycelial discs were then transferred to sterilised wheat grain and incubated. Tissue was cut from the fruiting bodies during each generation and cultured in new PDA plates. The processes of spawn run and cultivation were repeated for three generations namely F₁, F₂ and F₃. The cultivation data were recorded for the three generations.

ISSR ANALYSIS

Fruiting bodies of the parents and the hybrids strains (IH15, IH17, and IH32) were collected for analysis. Fruiting bodies were dried using food dehydrator for 24 h. DNA was extracted from dried fruiting body samples of all the parents and hybrids using E.Z.N.A® Forensic DNA Kit (Omega Bio-tek) following the manufacturer's protocol with slight modification. The purity and concentration of isolated DNA were determined using a NanoDrop 2000 spectrophotometer. Twenty arbitrary microsatellite primers (Bioneer Corp, Daejeon, Korea) were pre-selected for PCR amplifications. ISSR amplification was carried out using *i-Taq*TM Plus DNA

Polymerase kit (iNtRON Biotechnology Inc.). Each reaction contained 1× *i-Taq*TM Plus PCR buffer, 0.2 mM each dNTP, 0.4 -unit *i-Taq*TM Plus DNA Polymerase, 1 μM primer (Table 1), 50 ng sample DNA template and appropriate amount of autoclaved distilled water to make up to final reaction volume of 20 μL. DNA template was replaced with autoclaved distilled water in the reaction as a negative control. The amplification conditions were as follows: An initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 45 to 52 °C for 45 s, and extension at 72

°C for 90 s, followed by a final extension for 15 min at 72 °C. PCR products were resolved on 1.8% agarose gel mixed with RedSafeTM nucleic acid staining solution. The experiment was carried out in biological duplicates. ISSR fragments were recorded as present (1) or absent (0) of homologous bands. Those fragments with smeared or weak bands were excluded from the analysis. Polymorphic information content (PIC) and marker index (MI) were calculated to assess the performance of each primer (Varshney et al. 2007).

TABLE 1. List of primers used for generation of ISSR data

Primer	Sequence (5'-3')	Annealing temperature (°C)
P1	ACACACACACACACACC	52
P2	ACACACACACACACACG	48
P4	AGAGAGAGAGAGAGAGC	45
P13	GGATGCAACACACACACAC	52
P16	GTGACACACACACAC	52
P17	TGCACACACACACAC	52

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) of means of triplicate values was performed using the statistical package for the Social Sciences (SPSS) statistical software version 22 followed by Duncan's multiple range test to determine if there was for significant differences ($p < 0.05$) between treatments.

RESULTS

MATING STUDIES

Basidiospores of the parents took 7 to 10 days to germinate and produce the monokaryon cultures. A total of 14 fast-growing monokaryons of the parent KLU-M1227 were hybridised with 4 monokaryons isolated from the wild parent KLU-M1391. Sixteen hybrids were obtained from 56 combinations with a success rate of 28.6%.

LINEAR GROWTH STUDY

Both parents and the 16 hybrids took 24 to 49 days to fully colonise the substrate in the race tube. Among the hybrids tested, 37.5% of them formed primordia in the race tube even before the mycelium had fully colonised the substrate. As shown in Figure 1, the fastest-growing hybrids based on the linear growth rate were IH15 and IH32. Both of the hybrids had a linear growth rate at 0.33 ± 0.00 cm/day. Meanwhile, the parents had a lower linear growth rate of 0.18 ± 0.00 cm/day (KLU-M1227) and 0.19 ± 0.00 cm/day (KLU-M1391), which were 83% and 73% lower than the generated hybrids, respectively. The hybrids were grouped as slow-growing (growth rate < 0.29 cm/day) and fast-growing (growth rate ≥ 0.30 cm/day) based on the median ($M = 0.29$) (Figure 1). All the tested hybrids grew at least 13% faster than the parents. However, hybrid strains IH15, IH17 and IH32 (growth

rate ≥ 0.30 cm/day) were selected for further cultivation evaluated based on susceptibility test (unpublished data). The selected hybrids have better features in mycelial stage namely fast mycelial growth rate and dense mycelium,

which are the criteria for relative improvement over parents (Abdulgani et al. 2017; Guadarrama-Mendoza et al. 2014; Sobal et al. 2007).

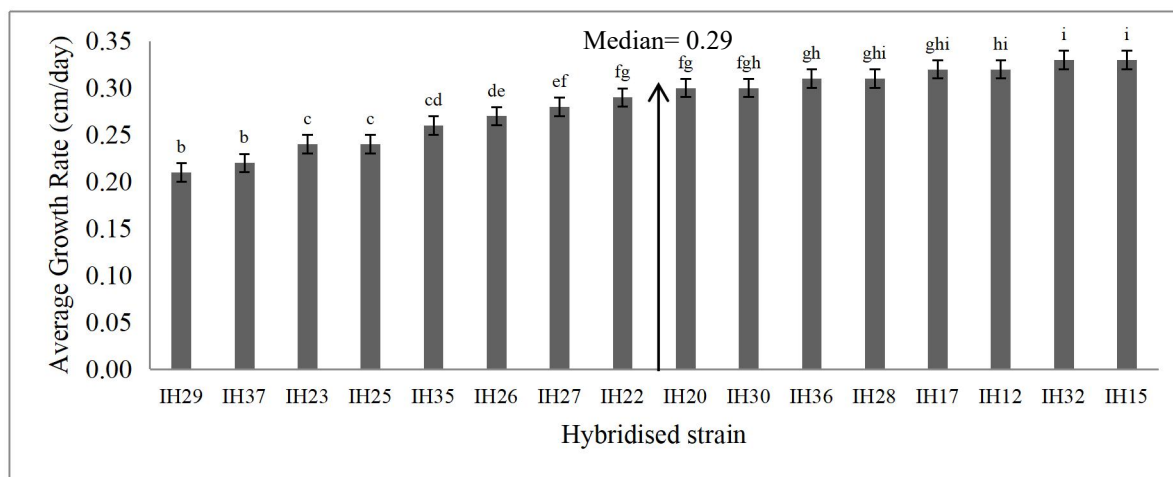


FIGURE 1. Average linear growth rate of hybrids of *Pleurotus giganteus* at 26 ± 2 °C in dark condition [substrate: 89% (w/w) sawdust, 10% (w/w) rice bran, 1% (w/w) calcium carbonate]. Average linear growth rate of the parent KLU-M1227 was 0.18 ± 0.01 a cm/day and the parent KLU-M1391 was 0.19 ± 0.01 a cm/day. Different letters indicate significant differences by Duncan's multiple range test ($p < 0.05$)

CULTIVATION OF THE PARENTS AND SELECTED HYBRIDS USING CONVENTIONAL CULTIVATION AND SOIL-CULTIVATION METHODS

Cultivation of the parents and selected hybrids (IH15, IH17, and IH32) was initially carried out using conventional method. Soil-casing method was then adopted to compare the yield as well as the physiological characteristics of the mushrooms of the conventional cultivation method. All the cultivated parents and hybrids were cased with soil 1 week after complete mycelium colonisation of the substrate.

For both cultivation methods, time required for the completion of mycelium colonisation of the substrate by the parents and hybrids of *P. giganteus* took 2 to 4 weeks (Table 2). During the conventional cultivation, the parent KLU-M1391 formed pinheads 2 weeks after spawn run, which was three times faster than the hybrid IH15 whereas in the soil-casing method, the parent KLU-M1227 was the fastest to produce pinheads compared to the wild parent and hybrids. It took 7 days to form pinheads after soil-casing, which was three times faster than the hybrid IH17 (Table 2).

TABLE 2. Fruiting body production of *P. giganteus* using conventional and soil-casing cultivation methods

Strain	Number of days for spawn completion		Number of days to show pinhead		No. of fruiting bodies		Biological efficiency (%)		Total no. of flushes	
	Conventional	Soil-casing	Conventional	Soil-casing	Conventional	Soil-casing	Conventional	Soil-casing	Conventional	Soil-casing
KLU-M1227	26.3 \pm	28.0 \pm	28.3 \pm	7.3 \pm	1.6 \pm	3.0 \pm	11.8 \pm	10.1 \pm	1.0 \pm	2.3 \pm
	1.6 ^c	0.0 ^c	8.3 ^{abc}	1.3 ^a	0.3 ^a	1.0 ^{ab}	1.8 ^{ab}	1.2 ^{ab}	0.0 ^{ab}	0.3 ^c
KLU-M1391	24.0 \pm	23.0 \pm	15.6 \pm	12.0 \pm	0.6 \pm	6.0 \pm	5.9 \pm	40.4 \pm	0.3 \pm	2.0 \pm
	1.0 ^{bc}	0.0 ^{bc}	15.6 ^{abc}	2.0 ^{abc}	0.6 ^a	0.5 ^b	5.9 ^a	1.8 ^c	0.3 ^a	0.0 ^c

IH15	16.0±	16.0±	47.3±	19.0±	1.6±	3.6±	28.1±	41.5±	1.0±	2.0±
	1.5 ^a	1.5 ^a	6.6 ^c	1.7 ^{abc}	0.6 ^a	1.6 ^{ab}	2.8 ^c	1.9 ^c	0.0 ^{ab}	0.0 ^c
IH17	18.3±	16.3±	46.0±	20.3±	2.1±	4.0±	24.3±	37.9±	1.0±	2.0±
	0.3 ^{ab}	0.3 ^a	23.1 ^{bc}	4.9 ^{abc}	1.4 ^{ab}	1.0 ^{ab}	12.8 ^{bc}	2.9 ^c	0.5 ^{ab}	0.0 ^c
IH32	16.0±	14.0±	26.6±	10.0±	2.8±	11.0±	36.2±	39.8±	1.6±	2.0±
	5.0 ^a	0.0 ^a	15.2 ^{abc}	0.0 ^{ab}	0.7 ^{ab}	2.4 ^c	6.5 ^c	0.6 ^c	0.3 ^{bc}	0.0 ^c

Values are mean ± standard error of three replicates. Values in columns followed by different letters indicate significant differences by Duncan's multiple range test ($p < 0.05$)

Long coiling stipes were formed by all the cultivated parents and hybrids in the mushroom bags using conventional cultivation method (Figure 2(C) i-ii). After harvesting, the remaining stipes inside the mushroom bags attracted insects and this eventually led to bag contamination. The parents formed 1 to 2 fruiting bodies whereas the hybrids formed 2 to 3 fruiting bodies on average. In contrast, no long coiling stipe was formed during the fruiting of *P. giganteus* for the soil-casing cultivation method. All of the cultivated parents and hybrids had short stipes in the soil-cased substrate bags (Figure 2(D)). In the soil-casing cultivation, the parents formed 3 to 6 fruiting bodies on average whereas the hybrids formed 4 to 11 fruit bodies.

In Table 2, all the hybrids had at least two times higher biological efficiency compared to the parents in conventional cultivation method whereas in soil-casing method, the parent KLU-M1227 showed the lowest biological efficiency, which was only 10%. However, the parent KLU-M1391 had the second highest biological efficiency compared to all other cultivated parent and hybrids. The biological efficiency of the hybrids was 38 to 42%. The crop of *P. giganteus* harvested in 1 to 2 flushes during conventional cultivation method while 2 flushes on an average were obtained in the soil-casing method. Overall, *P. giganteus* with soil-casing took shorter time for pinhead formation and formed higher number of fruiting body, higher biological efficiency as well as higher number of flushes compared to the fruiting body production using conventional cultivation method.

Among the parents and hybrids, there was no distinct variation in terms of morphological characteristics such as the colour of fruiting body and pileus margin for both cultivation methods (Figure 2(B), 2(E), 2(F)). For all cultivated strains, the pilei were regular and even, slightly depressed and the pilei surfaces were smooth with scales at the margin. The pilei margins were slightly uplifted in over-matured mushrooms. The pilei colour was

yellowish brown (5E5) to dark brown (6F7) in the center, towards the margin yellowish white (4A2) to light brown (6D6). The lamella was pale yellowish white (2A2), sub-distant spacing, de-current with narrow breadth. The stipe was 47 to 90 mm in length, 3 to 8 mm thick, and the colour varied from greyish brown (6F3) to orange white (5A2), with solid interior and fibrous texture.

A major problem encountered throughout the period of cultivation (both conventional and soil-casing methods) was the parent KLU-M1227 forming primordia after an average of 18 days but remained as pinhead for a long period without differentiating into pileus. The pinheads were attacked by green mold after 1 month. However, the parent KLU-M1227 was able to differentiate into a relatively small but deformed pileus (Figure 2(A)) when the room temperature was 23 to 32 °C (night–day) and humidity was 27 to 99% (day–night). When the room temperature was in the range of 22 to 35 °C (night–day) and humidity was 18 to 99% (day–night), the parent KLU-M1227 did not form fruiting bodies. Among the parents and selected hybrids investigated, the parent KLU-M1227 was the only strain with malformation of mushroom pileus (Figure 2(A)).

During the incubation period, though the mushroom house had two humidifiers, there were distinct changes in the external environmental conditions, the temperature of the mushroom house varied at 23 to 38 °C while the humidity at 14 to 99% (day–night). These variations resulted in a long period of 'mushroom hibernation' as the primordia were not formed in the conventional method without casing layer. In contrast, substrate cased with the soil layer showed shorter period for pinhead emergence even under such inconsistent circumstances because the soil layer served as a reservoir for water retention.

Since soil-casing cultivation performed better results in all aspects particularly biological efficiency and total number of flushes, this method was carried out for the parents and all the hybrids with 10 replicates each and for three generations.

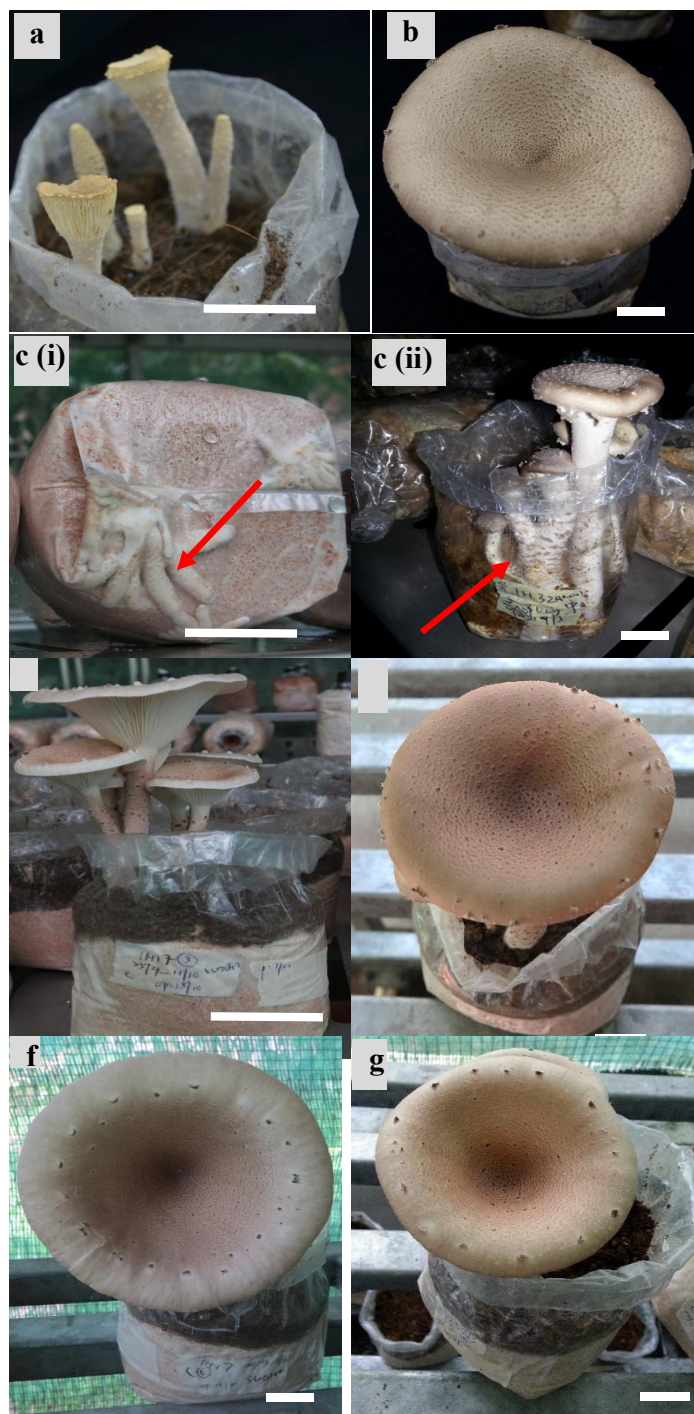


FIGURE 2. Fruiting bodies of the *Pleurotus giganteus*. a: Parent, KLU-M1227; b: Parent, KLU-M1391; c: Conventional cultivation method; (i) long stipe formed at the bottom of mushroom bag; (ii) long stipe formed by fruiting bodies. d: Absence of long stipe using soil-casing cultivation method; e: Hybrid, IH15; f: Hybrid, IH17; g: Hybrid, IH32. Bars: 20 mm

CULTIVATION OF THE PARENTS AND HYBRIDS OF *P. giganteus* USING SOIL-CASING CULTIVATION METHOD

As shown in Table 3, both the parents took 24 to 27 days for spawn completion in the F₁ generation trials while the hybrids took 2 weeks to fully colonise the substrate in mushroom bags. The parent KLU-M1227, however, was the fastest to produce pinhead among the parents and all the hybrids tested. The pinhead formation of KLU-M1227 was three times faster than that of IH17. The parent KLU-M1227, however, formed the lowest number of fruiting bodies (1.1±0.5) among the strains. Meanwhile, IH32 formed the highest number of fruiting bodies (6.8±1.7), which was ten times higher than the primordial formed by KLU-M1227. The parents and hybrids had 1 to 2 flushes on average. Due to the retarded

growth, KLU-M1227 formed the smallest pileus (1.0±0.4 cm) among all the cultivated *P. giganteus*. The pileus size of IH15 (7.2±0.4) and IH17 (7.2±0.3) was seven times larger than that of KLU-M1227. The stipe thickness of all the parents and hybrids was 0.5 to 0.9 cm, while the stipe length was 2.8 to 6.4 cm. During the F₁ generation trials (temperature was 23 to 35.4 °C and humidity was 39 to 99%), successful fruiting by all hybrids was obtained except 60% of the parent KLU-M1227 and 10% of the parent KLU-M1391 did not form fruiting bodies. The biological efficiency of the parent KLU-M1227 was the lowest (3.73%) among the parents and all the hybrids evaluated (Figure 3), while the hybrid IH15 had the highest biological efficiency, which was 11 times higher than that of KLU-M1227.

TABLE 3. Comparison of the fruiting body production of the parents and hybrids of *P. giganteus* in three generations using soil-casing method

Strain	Generation	No. of days for spawn completion	No. of days for pinhead formation	No. of fruiting bodies	Total no. of flush	Pileus size (cm)	Stipe thickness (cm)	Stipe length (cm)
KLU-M1227	1	23.7±1.2 ^b	7.9±1.3 ^a	1.1±0.5 ^a	1.4±0.2 ^a	1.0±0.4 ^a	0.5±0.2 ^a	2.8±1.2 ^a
	2	19.1±0.8 ^a	19.8±5.9 ^a	2.0±0.2 ^a	1.7±0.1 ^a	2.8±0.1 ^b	2.3±0.8 ^b	7.7±1.0 ^b
	3	25.2±1.7 ^b	13.3±3.7 ^a	2.0±0.2 ^a	1.9±0.1 ^a	1.9±0.3 ^{ab}	0.9±0.1 ^{ab}	7.2±0.3 ^b
	Average	22.6±1.8 ^a	13.7±3.4 ^a	1.7±0.3 ^a	1.6±0.1 ^a	1.9±0.5 ^a	1.2±0.5 ^a	5.9±1.5 ^a
KLU-M1391	1	26.8±0.9 ^b	15.0±4.1 ^a	4.0±0.7 ^b	1.5±0.2 ^a	5.4±0.8 ^a	0.8±0.1 ^a	4.5±0.5 ^a
	2	17.9±0.3 ^a	21.1±2.7 ^{ab}	3.8±0.5 ^b	2.1±0.2 ^{ab}	7.6±0.4 ^b	0.8±0.0 ^a	5.9±0.2 ^b
	3	18.5±1.3 ^a	32.7±6.1 ^b	1.8±0.1 ^a	2.7±0.2 ^b	7.2±0.3 ^b	0.9±0.0 ^a	6.4±0.1 ^b
	Average	21.0±2.8 ^a	22.9±5.1 ^{ab}	3.2±0.7 ^a	2.1±0.3 ^a	6.7±0.6 ^b	0.8±0.0 ^a	5.6±0.5 ^a
IH15	1	16.5±0.4 ^a	17.2±1.3 ^a	5.5±0.8 ^b	1.9±0.1 ^a	7.2±0.4 ^a	0.8±0.0 ^a	6.4±0.3 ^a
	2	19.7±0.5 ^b	20.4±3.6 ^a	2.0±0.4 ^a	2.0±0.3 ^a	6.6±1.2 ^a	0.8±0.1 ^a	5.8±0.9 ^a
	3	20.3±0.9 ^b	45.6±3.4 ^b	1.9±0.2 ^a	2.0±0.1 ^a	8.4±0.6 ^a	0.9±0.0 ^a	7.7±0.5 ^a
	Average	18.8±1.1 ^a	27.7±8.9 ^{ab}	3.1±1.1 ^a	1.9±0.0 ^a	7.4±0.5 ^b	0.8±0.0 ^a	6.6±0.5 ^a
IH17	1	16.7±0.3 ^a	25.8±5.4 ^a	4.4±0.7 ^a	1.8±0.1 ^a	7.2±0.3 ^a	0.9±0.0 ^b	6.0±0.2 ^a
	2	17.4±1.1 ^a	59.3±4.5 ^c	3.6±0.4 ^a	1.7±0.1 ^a	10.3±2.4 ^a	1.0±0.0 ^b	5.4±0.1 ^a
	3	21.6±1.3 ^b	45.6±3.7 ^b	2.8±0.2 ^a	1.8±0.2 ^a	6.6±0.3 ^a	0.8±0.0 ^a	5.9±0.3 ^a
	Average	18.5±1.5 ^a	43.5±9.7 ^b	3.6±0.4 ^{ab}	1.7±0.0 ^a	8.0±1.1 ^b	0.9±0.0 ^a	5.8±0.1 ^a
IH32	1	14.9±0.5 ^a	10.0±0.4 ^a	10.3±1.2 ^b	2.0±0.0 ^a	5.3±0.3 ^a	0.7±0.0 ^a	5.1±0.1 ^a
	2	17.2±1.1 ^a	6.9±1.1 ^a	5.4±0.8 ^a	2.4±0.1 ^a	7.1±0.5 ^b	0.9±0.0 ^b	6.1±0.2 ^b
	3	23.9±1.8 ^b	20.5±4.4 ^b	4.7±1.0 ^a	2.4±0.2 ^a	6.9±0.2 ^b	0.7±0.0 ^a	6.3±0.3 ^b
	Average	18.6±2.6 ^a	12.4±4.1 ^a	6.8±1.7 ^b	2.2±0.1 ^a	6.5±0.5 ^b	0.8±0.0 ^a	5.8±0.3 ^a

Values are mean ± standard error of ten replicates. Values in columns followed by different letters indicate significant differences by Duncan's multiple range test ($p < 0.05$)

In the second generation evaluation, the parents and all the hybrids showed no considerable difference in the number of days for spawn completion, which was 17 to 19 days. The hybrid IH32 formed pinhead 1 week after soil-casing, which was three times faster than both the parents and the hybrid IH15, but eight times faster than the hybrid IH17. The parent KLU-M1227 and the hybrid IH15 formed 2 fruiting bodies on average during F₂ generation. IH32 formed the highest number of fruiting bodies (5.4±0.8) among the parents and all the hybrids. The parents and hybrids had 2 flushes on average. IH17 formed the largest pileus (10.3±2.4 cm) among all the

cultivated samples, which was around three times of the pileus size of the parent KLU-M1227. Despite the small pileus size, KLU-M1227 had the thickest stipes (2.3±0.8 cm), which was two times thicker than all the others. KLU-M1227 also formed the longest stipe (7.7±1.0 cm) among them. During the F₂ generation (temperature was 20 to 38 °C and humidity was 14 to 99%), all parents and hybrids fruited except 20% of the hybrid IH15. Biological efficiency of IH32 was the highest among all the hybrids (56.79%), which was four times higher than that of KLU-M1227 (Figure 3).

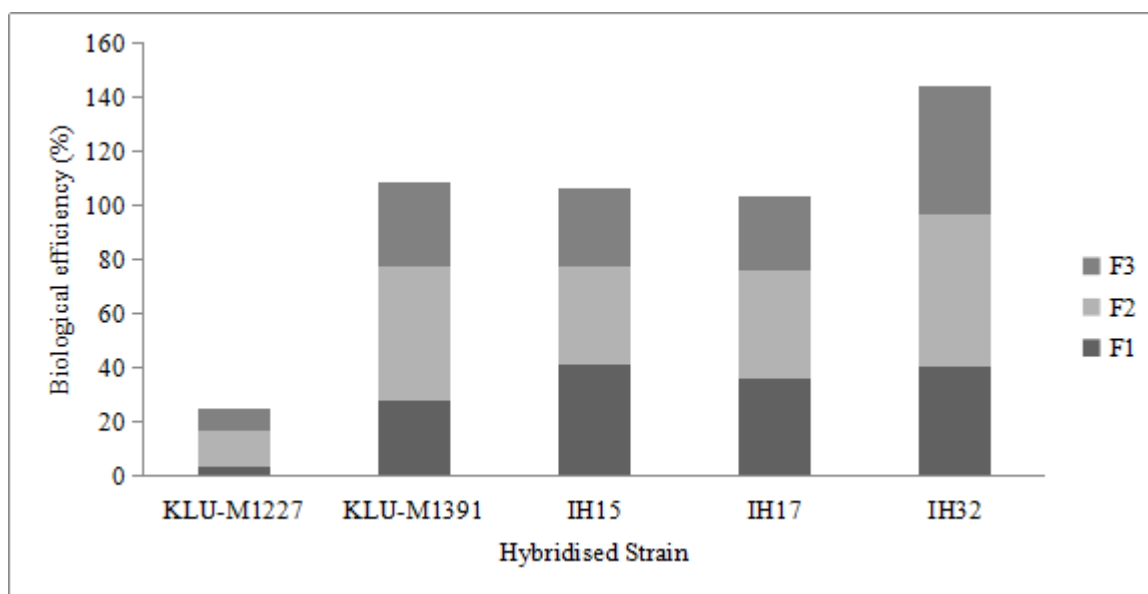


FIGURE 3. Biological efficiency (%) of the parents and selected hybrids in three generations

The parent KLU-M1391 took the shortest time for spawn completion among all the cultivated *P. giganteus* in the third generation (F₃) trials. While the parent KLU-M1227 was the slowest to complete spawn run in the mushroom bags. All the hybrids took around 3 weeks for spawn completion. IH32 was the fastest to show pinhead after soil-casing among all the hybrids. It took 20.5 days for pinhead formation, which was 55% faster than those of IH15 and IH17. Both the parents formed only 2 fruiting bodies in this generation whereas the number of fruiting bodies formed by the hybrids was 2 to 5. The parents and hybrids had 2 to 3 flushes on average. IH15 formed the largest pileus (8.4±0.6 cm) among all the cultivated mushrooms, which was four times larger than that of KLU-M1227. All of the cultivated mushrooms formed similar stipe thickness in this generation, ranging from 0.7 to 0.9 cm. KLU-M1227 and IH15 formed the longest stipes among all the cultivated *P. giganteus*,

which were 7.2±0.3 cm and 7.7±0.5 cm, respectively. During the F₃ generation where the temperature was 21.5 to 35.7 °C and humidity was 14 to 99%, all of the *P. giganteus* fruited. IH32 showed the highest biological efficiency (47.12%) among all the hybrids, and it was six times higher than that of the parent KLU-M1227 (Figure 3).

Generally, the cultivated parents and all of the hybrids took a longer period for spawn completion compared to the first and second generations except for the parent KLU-M1391. However, they all produced the highest number of flushes in the F₃ generation (Table 3). All the parents and hybrids took a shorter period for pinhead formation during the first generation except for IH32.

All the hybrids took an average of 18 days for spawn completion, which was 12% and 18% faster than KLU-M1391 and KLU-M1227, respectively (Table 3).

Variability in time to form pinhead was observed among the strains. Among the hybrids, IH32 was the fastest to fruit, which differed significantly ($p < 0.05$) from the other strains. Pinhead of hybrid IH32 grew at an average of 12.4 ± 4.1 days after bag opening while IH15 and IH17 took longer period for pinhead formation compared to both of the parents KLU-M1227 and KLU-M1391. The parent KLU-M1227 and KLU-M1391 took only 13.7 ± 3.4 and 22.9 ± 5.1 days, respectively, whereas the hybrids IH15 and IH17 had taken 27.7 ± 8.9 and 43.5 ± 9.7 days, respectively, to form pinhead after bag opening.

Hybrid IH32 produced the highest number of fruiting bodies compared to all other parents and hybrids in all three generations. The average number of fruiting body formed by IH32 (6.8 ± 1.7) was four times of that of the parent KLU-M1227 (1.7 ± 0.3) and two times of that of the parent KLU-M1391 (3.2 ± 0.7). The parents and all the hybrids produced an average of 1 to 2 flushes in the three generations.

The pileus size of the cultivated mushrooms was negatively correlated with the number of fruiting bodies formed whereby the mushroom bags with higher number of fruiting bodies formed smaller pileus. Even though hybrid IH32 had the highest number of fruiting body formed during the cultivation, its pileus size (6.5 ± 0.5 cm) was relatively small when compared to the other hybrids. The average pileus size of the cultivated strains was 6 - 8 cm, except for parent KLU-M1227 which showed retarded growth with an average of 1.9 ± 0.5 cm for pileus size (Table 3). However, KLU-M1227 showed the highest value in stipe thickness (1.2 ± 0.5 cm) among all the

cultivated strains despite its retarded pileus development.

The hybrid IH32 had the highest biological efficiency among all the cultivated strains throughout all three generations with a mean value of 48.07%. When comparing the fruiting body production among all strains within the three generations, the mushroom yield and biological efficiency during F_2 generation were generally higher (Figure 3). The biological efficiency of IH32 during the F_2 generation was 335% higher than the parent KLU-M1227 and 14% higher than the parent KLU-M1391. Among all the strains, KLU-M1227 showed the least percentage in biological efficiency due to its retarded growth. This parent performed only 3.73 ± 1.58 (%) of biological efficiency during the F_1 generation. The mean value of the biological efficiency of the hybrids in this study was 34.4 to 48.07%. However, the hybrid with the best yield (IH32) performed 479.66% and 32.35% higher in biological efficiency respectively when compared to the parents, KLU-M1227 and KLU-M1391.

MOLECULAR GENETIC ANALYSIS BY ISSR

Six ISSR primers produced a total of 30 loci with the size ranging from approximately 350 to 1500 bp. ISSR P13 produced 7 bands ranging from around 600 to 1500 bp for the parents and hybrids (IH15, IH17, and IH32). PIC values for primers ranged from 0.21 to 0.44 while MI values ranged from 0.61 to 1.48. Fragment patterns of IH17 and IH32 were shown to possess bands specific to KLU-M1227 and KLU-M1391 (Figure 4). Interestingly, IH15 shared all the bands (22) with KLU-M1391 and only shared 7 bands with KLU-M1227 (Table 4).

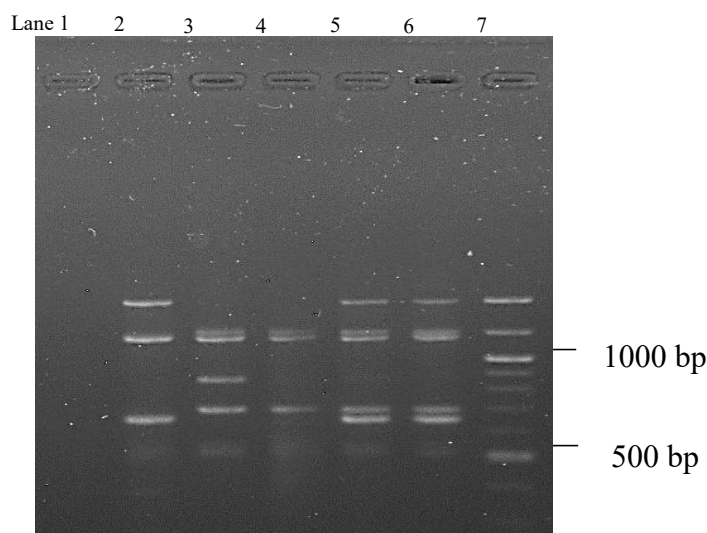


FIGURE 4. Inter simple sequence repeat (ISSR) profile of two parents and hybrids amplified by primer P17. Lane 1 = Negative control; Lane 2 = KLU-M1227 (parent); Lane 3 = KLU-M1391 (parent); Lane 4 = Hybrid IH15; Lane 5 = Hybrid IH17; Lane 6 = Hybrid IH32; Lane 7 = DNA ladder

TABLE 4. Band shared among all samples

Suspected hybrid	Bands shared with PG 001	Bands shared with PG 003	Bands shared with both parents	Bands for hybrid only
IH15	7 (31.8%)	22 (100%)	7 (31.8%)	0 (0%)
IH17	13 (48.1%)	19 (70.4%)	6 (22.2%)	0 (0%)
IH32	13 (59.1%)	16 (72.7%)	6 (27.3%)	0 (0%)

DISCUSSION

This study involved the crossing of basidiospores isolated from commercial and wild strains. This is a common breeding strategy which incorporates quantitatively inherited traits of a wild line into an existing commercial line (Sonnenberg et al. 2005). The mating frequency in this study was 3.6% higher than the expected mating frequency of tetrapolar basidiomycetes reported by Giraud et al. (2008), which was 25%. However, other studies showed higher mating frequency in tetrapolar basidiomycetes such as *P. tuber-regium* with 55 to 84% mating frequency reported by Isikhuemhen et al. (2000), 92% of mating frequency resulted by intraspecific mating of *P. ostreatus* reported by Kumara and Edirimanna (2009), and 26.3% of mating compatibility of *Hypsizygus marmoreus* reported by Lee et al. (2011). In the taxonomic study by Stajić et al. (2005), geographical isolation was said to cause genetic differences in *Pleurotus* strains. van Peer et al. (2011) also added that geographical distribution caused differences on the multi-allelic nature of mating genes. However, Isikhuemhen et al. (2000) and Lee et al. (2011) showed that tetrapolar mating using intrastock pairings from different geographical origins could achieve high mating compatibility frequency up to 70% or more. Therefore, there is possibility that the mating frequency in this study can be improved by increasing the number of basidiospores from the parental strains. Larsen et al. (1992) reported that clamp connections have become important as an *in vitro* indicator for dikaryon formation. Nuclei exchange and migration resulted in clamp cell formation (Brown & Casselton 2001). Following nuclear division and septation, clamp cell fusion occurs, and this results in a sexually competent dikaryon (Brown & Casselton 2001; Gharehaghaji et al. 2007).

Linear growth study was carried out to compare the mycelial growth rate between hybrids using a substrate composition commonly used in mushroom farms in Malaysia. According to Kumara and Edirimanna (2009), the genetic differences inherited within different strains may contribute to variation in mycelium colonisation rate

of a medium. In this study, the hybrids showed significant ($p < 0.05$) differences in linear growth rate but no variation was observed in the mycelium density on the substrate. This observation was similar to that reported by Mensah and Obodai (2014) that not all the fast-growing strains showed dense mycelium growth. Furthermore, Mensah and Obodai (2014) reported that mycelia growth rate and density is dependent on the media used. Since the linear growth is closely related to the yield of mushroom (Kaur et al. 2014), the linear growth rate in this study was not dependant on the mycelium density and should be taken into consideration when selecting the criteria of the substrate.

The morphological characteristics of the cultivated parents and all the hybrids of *P. giganteus* matched the descriptions by Karunarathna et al. (2011). Anyhow, environmental factors such as temperature, light intensity and carbon dioxide concentration may alter morphological characteristics of fungi (Bao et al. 2004). It was reported that factors such as inadequate light and ventilation, chemical vapours and overheated substrate during spawn run might lead to malformed fruiting bodies (Royse 2003). The abnormal maturation of the parent KLU-M1227 in this study could be related to high sensitivity of the strain and high temperatures experienced in this tropical climate. Apart from that, degeneration of this strain could also be due to genetic alteration and changes in environmental condition.

During soil-casing cultivation, the soil was sterilised before it was used for the casing layer. Cho et al. (2008) reported that compared to sterilised casing layer, non-sterilised casing layer contains more microorganisms which help to promote the formation of fruiting bodies. However, in Malaysia, the tropical climate with high humidity favours the growth of contaminants such as *Trichoderma* sp. Thus, non-sterilised casing layer may be a suitable habitat for unwanted microorganisms. This should be taken into considerations for the study of cultivation method using soil-casing layer. The pH value of the soil used in this study was pH 5. Mycelium started to colonise the soil layer 2 days after the substrate was

cased, this is in accordance with Klomklung et al. (2014) and Kumla et al. (2013) that mycelial colonisation of *P. giganteus* was optimal at pH 5.

Soil layer functioned as a medium for humidity retention. The cultivation method using soil-casing showed that the soil layer promoted the growth of primordium in the substrate in a shorter period, in agreement with previous reports which indicated that soil-casing helped in improving the growth of *Pleurotus* mushrooms and promoting primordial production of mushroom (Cho et al. 2008; Kumla et al. 2013). In China and Thailand, *P. giganteus* is cultivated using soil-casing method, which is said to be important for the fruiting stage to promote better yield (Klomklung et al. 2012).

This study was similar to the previous study by Kumla et al. (2013) who reported the pinhead formation after 2 weeks of incubation using soil-casing cultivation method. However, in this study, the cultivated *P. giganteus* parents and hybrids only took 3 to 5 days for the pinhead to develop into matured fruiting body. This result was contrast with the study by Kumla et al. (2013) who reported that the maturation of fruiting bodies of *P. giganteus* took 10 to 15 days.

Biological efficiency is an indication of the yield potential of a strain from a medium. In this study, hybrid IH 32 showed the highest biological efficiency among the cultivated strains. Hence, this hybrid had the highest capability to utilise the saw dust based medium compared to the other cultivated strains. The mean value of the biological efficiency of the hybrids in this study was 34.4 was 48.07%, which was considered slightly low if compared to the previous studies of *P. ostreatus* hybrid with biological efficiency 34.1 to 54.1% (Kumara & Edirimanna 2009). Cultivation is the most important stage to evaluate if the breeding study had produced improved strains that gave better yield and morphological characteristics such as larger pilei and better taste (Avin et al. 2016). Formation of fruiting body confirmed that there was a cross of the monokaryons isolated from both parents. In this study, an improved hybrid IH 32 had been produced as it showed higher biological efficiency, number of fruiting body and total number of flushes, as well as shorter period for spawn completion and pinhead formation in comparison to both parents. Cultivation of *P. giganteus* was carried out in small scale in this study. Thus, optimisation of the cultivation method of this species should be conducted on a large scale under farm conditions prior for commercialisation in Malaysia.

Morphological traits on vegetative and reproductive stages might not be sufficient as newly generated hybrids may have similar morphologies and difficult to be discriminated due to lack of clearly distinguishable characters. Molecular identification of newly generated hybrid is important in the mushroom breeding programme (Avin et al. 2016). Integration of morphological and molecular traits have been applied in the development of hybrid strain in edible mushroom (Avin et al. 2016;

Mallick et al. 2017). The ISSR analysis was carried out to assess genetic variations and differentiate hybrids and parents. However, hybrid IH15 shared most of the bands with the maternal strain, KLU-M1391, which could imply that most of the genetic materials from the hybrid might originate from single maternal strain (Guan et al. 2008). Furthermore, recombination might have occurred in the hybrid genome of IH17 and IH32 as both of the hybrids contained some of the parental bands which can be confirmed as newly developed hybrid.

CONCLUSION

This study showed that *P. giganteus* strains from different geographic regions, namely China and Malaysia were compatible and were mated successfully with a frequency of 28.6%. In this study, soil-casing cultivation method successfully solved the long coiling stipe formation problem of *P. giganteus*. This is the first mating study of *P. giganteus* in Malaysia. ISSR markers can help in detection of polymorphism among closely related genotype. In this study, hybrid IH17 and IH32 were shown to contain genetic materials from both parents while IH15 shared all loci with KLU-M1391 that could imply high amount of shared genetic materials.

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REFERENCES

- Avin, F.A., Bhassu, S., Ramech, V., Tan, Y.S. & Vikineswary, S. 2016. Genetics and hybrid breeding of *Pleurotus pulmonarius*: Heterosis, heritability and combining ability. *Euphytica* 209: 85-102.
- Abdulgani, R., Lau, C.C., Abdullah, N. & Vikineswary, S. 2017. Morphological and molecular characterization of *Pleurotus pulmonarius* hybrids with improved sporophore features and higher biological efficacy. *International Journal of Agriculture and Biology* 19(4): 707-712.
- Bao, D., Kinugasa, S. & Kitamoto, Y. 2004. The biological species of oyster mushrooms (*Pleurotus* spp.) from Asia based on mating compatibility tests. *Journal of Wood Science* 50: 162-168.
- Bi, Z., Zheng, G. & Li, T. 1993. *The Macrofungus Flora of China's Guangdong Province*. Hong Kong: The Chinese University Press.
- Bianco, C.L., Fernandez, J.A., Migliaro, D., Crino, P. & Egea-Gilabert, C. 2011. Identification of F1 hybrids of artichoke by ISSR markers and morphological analysis. *Molecular Breeding* 27: 157-170.
- Brown, A.J. & Casselton, L.A. 2001. Mating in mushrooms: Increasing the chances but prolonging the affair. *Trends Genetics* 17(7): 393-400.
- Castellani, A. 1968. Long term maintenance and cultivation of the common pathogenic fungi of man in sterile distilled water. *Pontificia Academia Scientiarum* 17: 1-8.

- Cho, Y.S., Weon, H.Y. & John, J.H. 2008. Effect of casing layer on growth promotion of the edible mushroom *Pleurotus ostreatus*. *Mycobiology* 36(1): 40-44.
- Dong, H.X., Cai, D.H. & Li, Y. 2010. Research situation and prospect of *Panus giganteus*. *Edible Fungi China* 29: 3-6.
- Doroteo, L.A., Segura, P.B.Z., Arce, R.V., Fernandez, J.Y., Aguilar, M.E.G., Guadarrama, M. & del Toro, G.V. 2018. Use of ITS and ISSR markers in the molecular characterisation of *Pleurotus djmor* hybrid strains. *Revista Iberoamericana de Micología* 35(1): 49-55.
- Gharehaghaji, A.N., Goltapeh, E.M. & Masiha, S. 2007. Hybrid production of oyster mushroom *Pleurotus ostreatus* (Jacq: Fries) Kummer. *Pakistan Journal of Biological Sciences* 10(14): 2334-2340.
- Giraud, T., Yockteng, R. & Villavicencio, M.L. 2008. Mating system of the anther smut fungus *Microbotryum violaceum*: Selfing under heterothallism. *Eukaryotic Cell* 7(5): 765-775.
- Gradzielewska, A., Gruszecka, D., Leśniowska-Nowak, J. & Paczos-Grzęda, E. 2012. Identification of hybrids between triticale and *Aegilops juvenalis* (Thell.) Eig and determination of genetic similarity with ISSRs. *Genetics and Molecular Research* 11(3): 2147-2155.
- Guadarrama-Mendoza, P.C., del Toro, G.V., Ramírez-Carrillo, R., Robles-Martínez, F., Yáñez-Fernández, J., Garín-Aguilar, M.E., Hernández, C.G. & Bravo-Villa, G. 2014. Morphology and mycelial growth rate of *Pleurotus* spp. strains from the Mexican mixtec region. *Brazilian Journal of Microbiology* 45(3): 861-872.
- Guan, X.J., Xu, L., Shao, Y.C., Wang, Z.R., Chen, F.S. & Luo, X.C. 2008. Differentiation of commercial strains of *Agaricus* species in China with inter-simple sequence repeat marker. *World Journal of Microbiology and Biotechnology* 24: 1617-1622.
- Huang, Q.R., Xin, X.L., Yang, L.H. & Pan, Y.S. 2005. Study of the submerged culture of *Clitocybe maxima* mycelia and its effect on isolated skeletal muscular fatigue induced by electric stimulation in toad. *Food Science* 26: 86-90.
- Isikhuemhen, O.S., Moncalvo, J., Nerud, F. & Vilgalys, R. 2000. Mating compatibility and phylogeography in *Pleurotus tuberregium*. *Mycological Research* 104(6): 732-737.
- Karunarathna, S.C., Yang, Z.L., Raspé, O., Ko Ko, T.W., Vellinga, E.C., Zhao, R.L., Bahkali, A.H., Chukeatirote, E., Degreef, J., Callac, P. & Hyde, K.D. 2011. *Lentinus giganteus* revisited: New collections from Sri Lanka and Thailand. *Mycotaxon* 118: 57-71.
- Kaur, S., Kapoor, S. & Sodhi, H.S. 2014. Screening and evaluation of *Agaricus bisporus* (Lange) Sing. strains for temperature variability. *International Journal of Current Microbiology and Applied Sciences* 3(6): 120-127.
- Klomklung, N., Karunarathna, S.C., Hyde, K.D. & Chukeatirote, E. 2014. Optimal conditions of mycelial growth of three wild edible mushrooms from Northern Thailand. *Acta Biologica Szegediensis* 58(1): 39-43.
- Klomklung, N., Karunarathna, S.C., Chukeatirote, E. & Hyde, K.D. 2012. Domestication of wild strain of *Pleurotus giganteus*. *Sydowia* 64(1): 39-53.
- Kornerup, A. & Wanscher, J.H. 1963. *Methuen Handbook of Colour*. London: Methuen & Co Ltd.
- Kothe, E. 2001. Mating-type genes for basidiomycete strain improvement in mushroom farming. *Applied Microbiology and Biotechnology* 56(5-6): 602-612.
- Kumara, K.W. & Edirimanna, I.C.S. 2009. Improvement of strains of two oyster mushroom cultivars using dual culture technique. *World Applied Sciences Journal* 7(5): 654-660.
- Kumla, J., Suwannarach, N., Jaiyasen, A. & Bussaban, B. 2013. Development of an edible wild strain of Thai oyster mushroom for economic mushroom production. *Chiang Mai Journal of Science* 40(2): 161-172.
- Larsen, M.J., Banik, M.T. & Burdsall, Jr. H.H. 1992. Clamp connections in North American *Armillaria* species: Occurrence and potential application for delimiting species. *Mycologia* 84(2): 214-218.
- Lee, C.Y., Park, J.E., Lee, J., Kim, J.K. & Ro, H.S. 2011. Development of new strains and related SCAR markers for an edible mushroom, *Hypsizygus marmoreus*. *FEMS Microbiology Immunology* 327(1): 54-59.
- Lee, J., Kang, H.W., Kim, S.W., Lee, C.Y. & Ro, H.S. 2011. Breeding of new strains of mushroom by basidiospore chemical mutagenesis. *Mycobiology* 39(4): 272-277.
- Mallick, P., Chattaraj, S. & Sikdar, S.R. 2017. Molecular characterizations of somatic hybrids developed between *Pleurotus florida* and *Lentinus squarrosulus* through inter-simple sequence repeats markers and sequencing of ribosomal RNA-ITS gene. *3 Biotechnology* 7(5): 298-304.
- Mensah, D.L.N. & Obodai, M. 2014. Morphological characteristics of mycelia .growth of two strains of the indigenous medicinal mushrooms, *Lentinus squarrosulus* Mont. (Singer) on solid media. *African Journal of Agricultural Research* 9(23): 1753-1760.
- MOA. 2019. National Agro-Food Policy 2011-2020 (Dasar Makanan Negara 2011-2020). Bab 7 Memacu Pertumbuhan Pertanian Bernilai Tinggi. pp. 96-98. <https://moa.gov.my/penerbitan>. Accessed on 30 April 2019.
- Pegler, D.N. 1983. The genus *Lentinus*: A world monograph. London: Her Majesty's Stationary Office.
- Petersen, R.H. & Bermudes, D. 1992. Intercontinental compatibility in *Panellus stypticus* with a note on bioluminescence. *Persoonia* 14(4): 457-463.
- Phan, C.W., Wong, J.K., Tan, E.Y.Y., Tan, Y.S., Sathiya Seelan, J.S., Cheah, S.C. & Sabaratnam, V. 2019. Giant oyster mushroom, *Pleurotus giganteus* (Agaricomycetes): Current status of the cultivation methods, chemical composition, biological and health-promoting properties. *Food Reviews International* 35(4): 324-341.
- Phan, C.W., David, P., Tan, Y.S., Naidu, M., Wong, K.H., Kuppusamy, U.R. & Sabaratnam, V. 2014. Intrastrain comparison of the chemical composition and antioxidant activity of an edible mushroom, *Pleurotus giganteus*, and its potent neurotogenic properties. *The Scientific World Journal* 2014(1): 1-10.
- Phan, C.W., Lee, G.S., Macreadie, I. & Malek, S.N.A. 2013. Lipid constituents of the edible mushroom, *Pleurotus giganteus* demonstrate anti-*Candida* activity. *Natural Product Communications* 8(12): 1763-1765.
- Phan, C.W., Wong, W.L., David, P., Naidu, M. & Sabaratnam, V. 2012. *Pleurotus giganteus* (Berk.) Karunarathna & K.D. Hyde: Nutritional value and *in vitro* neurite outgrowth activity in rat pheochromocytoma cells. *BMC Complementary and Alternative Medicine* 12(1): 102-112.
- Reddy, M.P., Sarla, N. & Siddiq, E. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128(1): 9-17.

- Royse, D.J. 2003. *Cultivation of Oyster Mushrooms*. College of Agricultural Sciences, Agricultural Research and Cooperative Extension. The Pennsylvania State University.
- Sahoo, A.K., Mohapatra, K.B., Behera, B.B. & Jadhao, K. 2014. Phylogenetic analysis of high yielding strain of paddy straw mushroom (*Volvariella volvacea*) by using ISSR markers. *Middle East Journal of Scientific Research* 21(8): 1197-1202.
- Sobal, M., Martínez-Carrera, D., Morales, P. & Roussos, S. 2007. Classical characterization of mushroom genetic resources from temperate and tropical regions of Mexico. *Micologia Aplicada Internacional* 19(1):15-23.
- Sonnenberg, A.S.M., Baars, J.J.P., Hendrickx, P.M. & Kerigan, R.W. 2005. Breeding mushrooms: State of the art. *Acta Edulis Fungi* 12: 163-173.
- Stajčić, M., Sikorski, J., Wasser, S.P. & Nevo, E. 2005. Genetic similarity and taxonomic relationships within the genus *Pleurotus* (higher Basidiomycetes) determined by RAPD analysis. *Mycotaxon* 93: 247-255.
- Urbanelli, S., Della Rosa, V., Punelli, F., Porretta, D., Reverberi, M., Fabbri, A.A. & Fanelli, C. 2007. DNA-fingerprinting (AFLP and RFLP) for genotypic identification in species of the *Pleurotus eryngii* complex. *Applied Microbiology and Biotechnology* 74(3): 592-600.
- van Peer, A.F., Park, S.Y., Shin, P.G., Jang, K.Y., Yoo, Y.B., Park, Y.J., Lee, B.M., Sung, G.H., James, T.Y. & Kong, W.S. 2011. Comparative genomics of the mating-type loci of the mushroom *Flammulina velutipes* reveals widespread synteny and recent inversions. *PLoS ONE* 6(7): 1-13.
- Varshney, R.K., Chabane, K., Hendre, P.S., Aggarwal, R.K. & Graner, A. 2007. Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Science* 173(6): 638-649.
- Wakchaure, G.C. 2011. Production and marketing of mushroom: Global and national scenario. In *Mushrooms-Cultivation, Marketing and Consumption*, edited by Singh, M., Vijaym, B., Kamal, S. & Wakchaure, G.C. India: Directorate of Mushroom Research. pp. 15-22.
- Wong, W.L., Abdulla, M.A., Chua, K.H., Kuppusamy, U.R., Tan, Y.S. & Sabaratnam, V. 2012. Hepatoprotective effects of *Panus giganteus* (Berk.) corner against thioacetamide-(TAA-) induced liver injury in rats. *Evidence-Based Complementary and Alternative Medicine* 2012(170303): 1-10.
- Yin, Y., Liu, Y., Li, H., Zhao, S., Wang, S., Liu, Y., Wu, D. & Xu, F. 2014. Genetic diversity of *Pleurotus pulmonarius* revealed by RAPD, ISSR, and SRAP fingerprinting. *Current Microbiology* 68(3): 397-403.
- Zhang, Q., Xu, B., Liu, L. Yuan, Q., Dong, H., Cheng, X. & Lin, D. 2012. Analysis of genetic diversity among Chinese *Pleurotus citrinopileatus* Singer cultivars using two molecular marker systems (ISSRs and SRAPs) and morphological traits. *World Journal of Microbiology and Biotechnology* 28(5): 2237-2248.

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