1 **Breeding of a high-yield strain for commercial cultivation by crossing *Pholiota adiposa* and *P.*** 2 ***limonella***

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15 **Abstract**

16 *Pholiota adiposa* is an edible mushroom with excellent nutritional and medicinal properties.
17 However, fruiting body yields are low, and the commercial cultivation potential of this fungus is
18 limited. In the present study, 279 crossbred strains were obtained by mono-mono crossing of
19 monokaryotic strains derived from *P. adiposa* HS5 and *P. limonella* HS4. Ligninolytic enzymes
20 and mycelial growth rate were used as markers to screen the crossbred strains, and 18 were selected
21 for further analysis. Crossbred strain A10B4 displayed the highest yield, i.e., 165.91 ± 12.56 g per
22 bag, which was 31.34 g and 74.48 g more than that of strains HS5 and HS4, respectively. The
23 mycelial colonization time of A10B4 was 25.18 ± 1.33 days, which was 5.64 days shorter than that

of HS5. A10B4 was characterized by inter-simple sequence repeat molecular markers and antagonism tests. Differences in PCR products from parental and crossbred strains were observed. Therefore, the newly developed hybrid strain A10B4, named *P. adiposa-limonella* HS54 and having a high yield and desirable traits, might be suitable for commercial cultivation.

Introduction

Pholiota adiposa (Batsch) P. Kumm. is an edible mushroom that is widely distributed on dead poplars, willows, or birches in forested areas in China [1, 2]. It is also a lignin-degrading macrofungus with excellent nutritional and medicinal properties [2]. Compounds extracted from the fruiting bodies or mycelia of *P. adiposa* display a variety of important biological activities, such as antitumor [2-4], antioxidative [5], antimicrobial [6], and anti-HIV-1 [7] effects. *P. adiposa* has become popular not only in China but also in several regions of Asia, Europe, and North America, owing to its delicious taste and beneficial properties [7, 8].

Mushroom breeding involves various methods, but crossbreeding is considered the most efficient way to develop new, good quality strains of edible mushrooms [9-11]. Intra-species crossbreeding has been reported in *Sparassis latifolia*, *Pleurotus tuoliensis*, *P. eryngii* species etc [10-13], and inter-species crossbreeding has been reported between *P. eryngii* and *P. nebrodensis* etc [14]. High yield is a key benefit of crossbreeding. Most *P. adiposa* cultivars were domesticated from the wild and their yields are not very high, and reports on breeding of this mushroom are limited. In our previous work, strain *P. adiposa* HS5 (JZB2116005) [1], which displayed the highest biological efficiency (BE, $67.88 \pm 1.33\%$, with fruiting bodies in two flushes) and mycelial growth rate (MGR, $2.56 \pm 0.03\text{mm/d}$), was screened as one of five wild

strains in comparative studies. *P. limonella* (Peck) Sacc. strain HS4 demonstrated an approximately 1.5-fold higher MGR than HS5 and was closely related to *P. adiposa*. Therefore, to obtain a *P. adiposa* strain with high yield and MGR, we performed inter-species crossbreeding of these two strains.

In *Pholiota* breeding to date, no effective and rapid marker-assisted selection strategies, except for clamp connection, have been developed to evaluate crossbred strains with important agronomic traits at the mycelial growth stage. Laccase is a ligninolytic enzyme. Many edible fungi such as *Volvariella volvacea* [15], *Lentinula edodes* [16, 17], and *Pleurotus florida* [18] have been shown to secrete laccase. Laccase has been shown important to decolorization of Remazol Brilliant Blue R (RBBR) and to lignin degradation and fruiting-body formation [19]. The close relationships between laccase activity, induced primordium differentiation, and development of fruiting bodies were reported by Yang et al [20]. Sun and Xu used decolorization of RBBR by a ligninolytic enzyme as a marker to screen protoplast fusants, and the growth rates and biological efficiencies of new strains were higher than those of the parental strains [19, 21]. Therefore, RBBR offers an efficient approach to evaluating new fungal strains.

In this study, crossbreeding between *P. adiposa* and *P. limonella* was used to develop a new strain (*P. adiposa-limonella*) with high BE and MGR, with decolorization of RBBR by a ligninolytic enzyme used as a marker to screen crossbred strains.

Materials and methods

Strains and growth conditions

The fruiting bodies of *P. adiposa* HS5 and *P. limonella* HS4 were collected from Haidian and Changping districts, respectively, in Beijing, China, and then isolated by tissue culture. The strains

were cultured and maintained on potato dextrose broth at 25°C. When required, 1.5% (wt/vol) agar was added to the appropriate medium.

Single-spore isolation

The method was performed as previously described by Wang et al [13]. with minor modifications. Briefly, fruiting bodies were placed in Erlenmeyer flasks containing 100 ml of sterilized water to generate spore suspensions. One hundred microliters of spore suspension (about 1×10^3 spores/ml) was spread onto potato dextrose agar (PDA) plates and incubated at 25°C for five days for spore germination. Monokaryons originating from monospores were identified based on the absence of clamp connections on mycelia ascertained by microscopy (400× magnification).

PDA-RBBR plate screening and MGR assessment of monokaryons

The strains were transferred from PDA to PDA-RBBR (PDA medium supplemented with RBBR to the terminal concentration of 0.05 % (w/v) to screen for monokaryons. The degree of RBBR fading was designated 1, 2, 3, or 4. A higher number represented greater RBBR decolorization with a more active ligninolytic enzyme. The MGR was determined by observing the radial growth length of mycelia on PDA-RBBR plates.

Pairings between single-spore isolates

Inter-strain pairing experiments were performed using random single-spore isolates (SSIs) derived from strains *P. adiposa* HS5 and *P. limonella* HS4. Mating was conducted by placing mycelial blocks opposite a monokaryotic mycelium on PDA. Mycelium fragments were taken from the contact zone between the paired colonies and individually transferred to PDA plates for further incubation. When the colonies grew to 1.0-1.5 cm in radius, the mycelia were monitored using a microscope to identify dikaryotic hybrids by clamp connections.

Antagonistic activity tests

Antagonistic activity assays were performed as previously described, with minor modifications (Xiang et al., 2016). Briefly, two parental strains and the putative hybrids were co-cultured at 25°C in 9-cm PDA plates, with every two mycelial fragments placed 2 cm apart. Somatic incompatibility reactions were observed after incubation for 10 days.

Fruiting and cultivation methods

Substrates were prepared with 67% (w/w) water content containing 60% cottonseed hull, 18% sawdust, 15% wheat bran, 5% corn flour, 1% gypsum, and 1% lime and placed in polypropylene bags (17 cm × 33 cm × 0.04 cm) at a packing density of 1,000 g of substrate per bag. The bags were autoclaved at 121°C for 120 min. The inoculated bags were then maintained in a spawn running room at 23-25°C and 50-60 relative humidity under dark conditions. After a complete spawn run, mycelial differentiation was induced by stimulation at 0-5°C for 3-5 days. The bags were then transferred to a fruiting chamber that was maintained at 18 ± 2°C and a relative humidity of 80-90% with 12 h of illumination (300-600 Lux) for 15-20 days to obtain fruiting bodies. Each strain was subjected to three treatments, with 12 bags per treatment. Fruiting bodies in first flushes were harvested, yield was the weight of fresh mushrooms per bag, and the biological efficiency (BE) of each fungal strain was calculated based on the following formula: BE (%) = weight of fresh mushrooms harvested per bag/dry weight of cultivation substrates per bag × 100. Mycelial colonization time was calculated as number of days from inoculation to complete colonization of the substrate by the mycelium in the culture bag.

Inter-simple sequence repeat analysis

Inter-simple sequence repeat (ISSR) amplification was performed in 20-μl reaction volumes

containing 0.5 U of Taq DNA polymerase, 2.5 mmol/l MgCl₂, 0.2 mmol/l dNTPs, 0.75 µmol/l primer, and 50 ng template DNA. Amplification conditions were as follows: 94°C for 5 min; 35 cycles of 94°C for 30 s, 45°C for 45 s, and 72°C for 90 s; and a final extension for 7 min at 72°C. Primer sequences were as follows: P11, AGAGAGAGAGAGAGAGG, P856, ACACACACA CACACACYA, P23, GAGAGAGAGAGAGAGACT, P857, ACACACACAC ACACACYG

Statistical analysis

All data were statistically analyzed using SPSS PASW Statistics software version 18. All data were obtained in triplicate, and differences were determined by Duncan's multiple range testing. Statistical differences were considered significant at the 5.0% level ($P < 0.05$).

Results

Selection of *P. adiposa* HS5 and *P. limonella* HS4 monokaryons

One hundred and twenty-six and 103 monokaryons were isolated from *P. adiposa* HS5 and *P. limonella* HS4, respectively. Monokaryons were selected for further study based on analysis of mycelial growth rates and their abilities to decolorization of RBBR (Fig 1). For *P. limonella* HS4, the average MGR of 103 monokaryons was 1.77 mm/d in the first round of screening (data not shown), 21 monokaryons with a fading degree ≥ 3 and MGR ≥ 1.77 mm/d were selected during the second round of screening (Table 1). For *P. adiposa* HS5, the average MGR of 126 monokaryons was 1.66 mm/d in the first round of screening (data not shown), 22 monokaryons with a fading degree ≥ 3 and MGR ≥ 1.66 mm/d were selected during the second round of screening (Table 2).

Screening of crossbred strains

Crossbreeding was conducted by mating 22 monokaryons isolated from *P. adiposa* HS5 with

21 monokaryons isolated from *P. limonella* HS4, resulting in a total of 462 matings. Crossbred strains were screened for clamp connections within the contact zone under a microscope. The presence of clamp connections indicates the formation of a dikaryon [12]. A primary screening of these 279 putative crossbred strains was performed on RBBR-PDA (fading degree ≥ 3 and an MGR greater than that of HS5) and resulted in the identification of 55 crossbred strains for further study. A second screening of the 61 strains resulted in 12 putative crossbred strains with fading degree ≥ 3 and an MGR greater than that of HS5 (Table 3). To avoid losing strains with good traits, the top six strains with a high MGR and fading degree ≤ 2 were also selected for further study. All 18 putative hybrids showed strong somatic incompatibility reactions with both parental strains, so these 18 crossbred strains were retained for further study.

Fruiting body production from crossbred strains

The parental and 18 crossbred strains were cultured, and fresh weight, and mycelial colonization time were compared between the strains (Table 4). Among the 18 crossbred strains, A20B3, A4B6, A16B3, and A16B6 showed abnormal fruiting bodies, and A5B1, A20B12, A6B6, and A10B12 did not form fruiting bodies at all. The weight of the fresh fruiting body of parental strain HS5 in one bag was $134.57 \pm 5.45\text{g}$, which was 43.14 g more than that of HS4 (Table 4). The BEs of HS5 and HS4 were $40.78 \pm 1.65\%$ and $27.71 \pm 1.59\%$, respectively, in the first flush (Table 4). Two crossbred strains (A10B4 and A14B4) were more productive than parental strain HS5 (Fig 2), and five crossbred strains were more productive than parental strain HS4. The most productive strain was A10B4, but the difference in productivity between A10B4 and A14B4 was not significant. The mycelial colonization time of HS5 was $30.82 \pm 0.98\text{days}$, which was 6.1 days longer than HS4 (Table 4). Among the crossbred strains, A10B5, A10B4, and A1B18 displayed

the shortest mycelial colonization times at 24.36 ± 1.03 , 25.18 ± 1.33 , and 25.00 ± 1.18 days, respectively. Comprehensive comparisons of yield, mycelial colonization time indicated that A10B4 possessed the most desirable traits of all of the crossbred strains.

ISSR analysis

Crossbred strain A10B4 and the parental strains were analyzed by ISSR. Among the tested primers, four primers P11, P856, P23, and P857 were found to efficiently amplify the genomic DNA from all strains (Fig 3). The size of the polymorphic fragments obtained ranged from 200 bp to 5000 bp, and differences were observed in the number of bands obtained (Fig 3). The crossbred strain and two parental strains showed characteristic differences in the presence and absence of fragments. More than eight different fragments were amplified in crossbred strain A10B4 using the four primers. The results of PCR amplification indicated that A10B4 is a new hybrid strain and that differences in numbers of fragments are useful to identifying this strain.

Discussion

Cross-breeding using monokaryons was an efficient method to develop new strains. In this study, *P. adiposa* and *P. limonella* were crossbred to develop a new strain (*P. adiposa-limonella*) with high yield and MGR. The new high-yield crossbred strains obtained in this study should benefit future commercial cultivation efforts.

Dikaryotic mycelia form a clamp connection [12, 22], and in this study, mycelia were screened by detecting clamp connections under a microscope to identify dikaryotic hybrids. White rot fungi have an array of extracellular ligninolytic enzymes that synergistically and efficiently degrade lignin [21]. RBBR is an anthra-quinone-based dye with a structural resemblance to some polycyclic aromatic hydrocarbons [19]. Ligninolytic enzymes can decolorize RBBR. Sun et al.

(2014) reported that ligninolytic enzymes can be used as a screening marker for new species of edible fungi, resulting in a screening method that is simple and direct. Selecting crossbred strains is an important step in breeding fungi, and, to reduce work and increase efficiency, we used RBBR fading degree (representing ligninolytic enzyme activity) and MGR to screen new strains in this study. One hundred and twenty-six and 103 monokaryons were isolated from *P. adiposa* HS5 and *P. limonella* HS4, respectively, and these monokaryons were selected based on ligninolytic enzyme activity and MGR, thus reducing breeding time compared with that needed only with clamp connection. The parental strain *P. adiposa* HS5 showed a higher yield and greater ligninolytic enzyme activity than *P. limonella* HS4. Six hybrid strains with high MGRs but relatively low ligninolytic enzyme activities (fading degrees ≤ 2) were also selected for mushroom production, and strains A1B18, A1B17, A1B11, A1B12, and A1B6 displayed low BEs, in general, with ligninolytic enzyme activities positively correlated with yield. The morphological characteristics of crossbred strains showed that monokaryon A1 was more closely related to HS4 than HS5. Among the 18 crossbred strains, four strains produced abnormal fruiting bodies, and four strains did not form fruiting bodies at all. The reason may be that *P. adiposa* and *P. limonella* are different species, and some of the resulting hybrids strains were infertile. To our knowledge, this is the first report of hybridization between *P. adiposa* and *P. limonella*. Ten crossbred strains formed normal fruiting bodies (Table 4). Strain A10B4 displayed the highest BE, as well as the shortest mycelial colonization time. Therefore, it was selected as the highest performing of the crossbred strains. ISSR and antagonistic activity assays confirmed that A10B4 is a new strain. Because the morphological characteristics of A10B4 are more like *P. adiposa* HS5, this strain was named *P. adiposa-limonella* HS4.

200 Conclusion

201 In conclusion, a new crossbred strain, *P. adiposa-limonella* HS54, demonstrated favorable
202 traits in terms of yield and BE. From a commercial point of view, the new strain has a clear
203 advantage over the parental strains and suggests potential for future commercial cultivation of
204 *Pholiota*.

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218 References

- 219 1. Rong C, Song S, Niu Y, Xu F, Liu Y, Zhao S, et al. Selection of a highly productive strain of
220 *Pholiota adiposa*. Mycosphere. 2016;7 (2):226–35. <https://doi.org/10.5943/mycosphere/7/2/11>.
- 221 2. Hu Q, Wang H, Ng TB. Isolation and purification of polysaccharides with anti-tumor activity

222 from *Pholiota adiposa* (Batsch) P. Kumm. (higher Basidiomycetes). International journal of
223 medicinal mushrooms. 2012;14(3):271-84. <https://doi.org/10.1615/IntJMedMushr.v14.i3.40>
224 PMID: 22577977.

225 3. Zhang GQ, Sun J, Wang HX, Ng TB. A novel lectin with antiproliferative activity from the
226 medicinal mushroom *Pholiota adiposa*. Acta biochimica Polonica. 2009;56(3):415-21. PMID:
227 19636442.

228 4. Zou Y, Du F, Hu Q, Wang H. The structural characterization of a polysaccharide exhibiting
229 antitumor effect from *Pholiota adiposa* mycelia. Scientific reports. 2019;9(1):1724.
230 <https://doi.org/10.1038/s41598-018-38251-6> PMID: 30741980 PMID: 30741980.

231 5. Deng P, Zhang G, Zhou B, Lin R, Jia L, Fan K, et al. Extraction and in vitro antioxidant activity
232 of intracellular polysaccharide by *Pholiota adiposa* SX-02. Journal of bioscience and
233 bioengineering. 2011;111(1):50-4. <https://doi.org/10.1016/j.jbiosc.2010.08.004> PMID:
234 20801714.

235 6. Dulger B. Antimicrobial activity of the macrofungus *Pholiota adiposa*. Fitoterapia. 2004;75(3-
236 4):395-7. <https://doi.org/10.1016/j.fitote.2004.01.005> PMID: 15159005.

237 7. Wang CR, Zhou R, Ng TB, Wong JH, Qiao WT, Liu F. First report on isolation of methyl
238 gallate with antioxidant, anti-HIV-1 and HIV-1 enzyme inhibitory activities from a mushroom
239 (*Pholiota adiposa*). Environmental toxicology and pharmacology. 2014;37(2):626-37.
240 <https://doi.org/10.1016/j.etap.2014.01.023> PMID: 24572641.

241 8. Cho SM, Lee YM, Lee DH, Chun HK, Lee JS. Effect of a *Pholiota adiposa* extract on fat mass
242 in hyperlipidemic mice. Mycobiology. 2006;34(4):236-9. [https://doi.org/10.4489/](https://doi.org/10.4489/MYCO.2006.34.4.236)
243 MYCO.2006.34.4.236 PMID: 24039505.

- 244 9. Xiang X, Wang X, Bian Y, Xu Z. Development of crossbreeding high-yield-potential strains for
245 commercial cultivation in the medicinal mushroom *Wolfiporia cocos* (Higher Basidiomycetes).
246 Journal of natural medicines. 2016;70(3):645-52. <https://doi.org/10.1007/s11418-016-0995-z>
247 PMID: 27100524.
- 248 10. Kim MK, Ryu J-S, Lee Y-H, Kim H-R. Breeding of a long shelf-life strain for commercial
249 cultivation by mono–mono crossing in *Pleurotus eryngii*. Scientia horticultrae. 2013;162:265-
250 70. <https://doi.org/10.1016/j.scienta.2013.08.028>.
- 251 11. Sonnenberg AS, Baars JJ, Gao W, Visser RG. Developments in breeding of *Agaricus bisporus*
252 var. *bisporus*: progress made and technical and legal hurdles to take. Applied microbiology
253 and biotechnology. 2017;101(5):1819-29. <https://doi.org/10.1007/s00253-017-8102-2> PMID:
254 28130632.
- 255 12. Sou HD, Ryoo R, Ryu SR, Ka KH, Park H, Joo SH. Morphological and genetic characteristics
256 of newly crossbred cauliflower mushroom (*Sparassis latifolia*). J Microbiol. 2013;51(5):552-7.
257 <https://doi.org/10.1007/s12275-013-2666-z> PMID: 23800950.
- 258 13. Wang S, Zhao S, Huang Z, Yin L, Hu J, Li J, et al. Development of a highly productive strain
259 of *Pleurotus tuoliensis* for commercial cultivation by crossbreeding. Scientia Horticulturae.
260 2018;234:110-5. <https://doi.org/10.1016/j.scienta.2018.02.001>.
- 261 14. Xu M. Distance hybridization of *Pleurotus eryngii* and *P. nebrodensis*. Fujian agriculture and
262 forestry university master thesis. 2010. <https://doi.org/10.7666/d.y1748296>.
- 263 15. Chen S, Ge W, Buswell JA. Molecular cloning of a new laccase from the edible straw
264 mushroom *Volvariella volvacea*: possible involvement in fruit body development. FEMS
265 microbiology letters. 2004;230(2):171-6. [https://doi.org/10.1016/S0378-1097\(03\)00878-4](https://doi.org/10.1016/S0378-1097(03)00878-4)

- 266 PMID: 14757236.
- 267 **16.** Nagai M, Kawata M, Watanabe H, Ogawa M, Saito K, Takesawa T, et al. Important role of
268 fungal intracellular laccase for melanin synthesis: purification and characterization of an
269 intracellular laccase from *Lentinula edodes* fruit bodies. Microbiology. 2003;149(Pt 9):2455-
270 62. <https://doi.org/10.1099/mic.0.26414-0> PMID: 12949171.
- 271 **17.** Nagai M, Sato T, Watanabe H, Saito K, Kawata M, Enei H. Purification and characterization
272 of an extracellular laccase from the edible mushroom *Lentinula edodes*, and decolorization of
273 chemically different dyes. Applied microbiology and biotechnology. 2002;60(3):327-35.
274 <https://doi.org/10.1007/s00253-002-1109-2> PMID: 12436315.
- 275 **18.** Palvannan T, Sathishkumar P. Production of laccase from *Pleurotus florida* NCIM 1243 using
276 Plackett-Burman design and response surface methodology. Journal of basic microbiology.
277 2010;50(4):325-35. <https://doi.org/10.1002/jobm.200900333>. PMID: 20473960.
- 278 **19.** Xu J, Zhang J, Zhang W, Hu K. The novel role of fungal intracellular laccase: used to screen
279 hybrids between *Hypsizygus marmoreus* and *Clitocybe maxima* by protoplasmic fusion. World
280 journal of microbiology & biotechnology. 2012;28(8):2625-33.[https://doi.org/10.1007/s11274-](https://doi.org/10.1007/s11274-012-1072-8)
281 012-1072-8 PMID: 22806188.
- 282 **20.** Wu X, An Q, Dai Y. Physiological and biochemical characteristics of the *Pleurotus ostreatus*
283 CCEF89. Mycosystema. 2015;34(4): 621-31. <https://doi.org/10.13346/j.mycosystema.150055>.
- 284 **21.** Sun S, Li X, Ruan L, Zhang L, Hu K. A novel breeding strategy for new strains of *Hypsizygus*
285 *marmoreus* and *Grifola frondosa* based on ligninolytic enzymes. World journal of
286 microbiology & biotechnology. 2014;30(7):2005-13. [https://doi.org/10.1007/s11274-014-](https://doi.org/10.1007/s11274-014-1624-1)
287 1624-1. PMID: 24535613.

288 **22.** Rong C, Zhao S, Li D, Wang L, Wang S, Ma K, et al. Cloning of the A mating-type locus
 289 from *Lepista nuda* and characterization of its genetic structure. Current microbiology.
 290 2015;71(6):669-77. <https://doi.org/10.1007/s00284-015-0902-y>. PMID: 26330378.

291 **Conflict of interest**

292 The authors declare that they have no conflicts of interest.

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295 **Table 1. Mycelial growth rate and fading degree of *P. limonella* HS4 monokaryons**

Numbers	Monokaryon number	Mycelial growth rate (mm/d)	Fading degree
1	45	3.10±0.10	1
2	65	2.76±0.02	1
3	82	2.70±0.20	3
4	103	2.53±0.02	2
5	15	2.52±0.06	1
6	75	2.49±0.03	1
7	87	2.48±0.06	2
8	72	2.42±0.07	3
9	90	2.42±0.07	3
10	78	2.39±1.11	2
11	43	2.39±0.05	3
12	100	2.39±0.02	3
13	42	2.38±0.02	4
14	101	2.33±0.05	3
15	46	2.26±0.05	2
16	107	2.24±0.02	4
17	92	2.19±0.03	2
18	39	2.18±0.04	1
19	9	2.17±0.02	3
20	70	2.16±0.06	1
21	28	2.16±0.04	2
22	32	2.14±0.06	2
23	10	2.13±0.02	2
24	30	2.13±0.02	2
25	16	2.12±0.03	2
26	40	2.11±0.02	2
27	102	2.11±0.02	3

28	84	2.10±0.05	4
29	50	2.10±0.17	4
30	62	2.05±0.03	4
31	95	2.05±0.02	3
32	41	1.99±0.13	3
33	76	1.98±0.06	3
34	29	1.96±0.04	2
35	51	1.93±0.69	3
36	67	1.89±0.03	4
37	68	1.88±0.03	3
38	2	1.85±0.05	4
39	19	1.77±0.03	4
40	52	1.72±0.07	4
41	83	1.63±0.05	4
42	25	1.56±0.04	4
43	5	1.51±0.05	4
44	3	1.47±0.09	4
45	4	1.44±0.02	4
46	89	1.44±0.02	3
47	71	1.36±0.05	4

296 Bold letters represented the monokaryons selected for hybridization.

297 **Table 2. Mycelial growth rate and fading degree of *P. adiposa* HS5 monokaryons**

Numbers	Monokaryon number	Mycelial growth rate (mm/d)	Fading degree
1	100	2.77±0.06	3
2	61	2.51±0.14	3
3	60	2.47±0.06	4
4	79	2.38±0.16	3
5	3	2.31±0.06	2

6	43	2.28±0.07	2
7	76	2.19±0.11	4
8	17	2.09±0.14	3
9	139	2.07±0.05	3
10	32	2.01±0.06	2
11	29	1.97±0.11	4
12	11	1.95± 0.06	2
13	112	1.95±0.10	3
14	23	1.88±0.02	2
15	173	1.88±0.12	3
16	143	1.84±0.04	4
17	126	1.82±0.10	3
18	80	1.80±0.04	2
19	2	1.78±0.10	4
20	113	1.78±0.10	2
21	159	1.77±0.06	1
22	49	1.75±0.08	4
23	78	1.75±0.15	3
24	142	1.73±0.07	4
25	101	1.73±0.06	4
26	136	1.72±0.13	3
27	130	1.69±0.05	4
28	7	1.68±0.09	4
29	107	1.66±0.12	4
30	10	1.66±0.06	4
31	172	1.65±0.05	1
32	53	1.64±0.03	4
33	58	1.63±0.08	4
34	6	1.22±0.02	4
35	119	1.20±0.08	2

36	24	1.02± 0.30	2
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298 Bold letters represented the monokaryons selected for hybridization.

299 **Table 3. Mycelial growth rate and fading degree of crossbred and parental strains**

Numbers	Crossbred strains	Mycelial growth rate (mm/d)	Fading degree
Parental strain	HS4	3.46±0.10	1
Parental strain	HS5	2.29±0.17	2
1	A5B1	3.14±0.09	1
2	A1B18	2.81±0.16	2
3	A1B17	2.66±0.23	2
4	A1B11	2.57±0.12	2
5	A1B12	2.57±0.18	2
6	A1B6	2.53±0.12	2
7	A10B5	2.47±0.05	3
8	A16B3	2.46±0.05	3
9	A20B3	2.44±0.08	3
10	A13B4	2.43±0.03	2
11	A8B6	2.40±0.12	3
12	A17B21	2.38±0.06	2
13	A21B6	2.38±0.06	2
14	A4B6	2.36±0.12	3
15	A14B4	2.36±0.15	3
16	A10B4	2.35±0.03	3
17	A20B12	2.35±0.03	3
18	A8B9	2.34±0.08	2
19	A6B6	2.34±0.04	3
20	A13B6	2.31±0.08	2
21	A7B6	2.31±0.03	3
22	A10B12	2.30±0.08	4
23	A16B6	2.30±0.05	4

24	A11B2	2.29±0.06	3
25	A14B21	2.29±0.04	3
26	A4B5	2.27±0.07	3
27	A21B12	2.27±0.05	4
28	A13B21	2.26±0.07	3
29	A10B10	2.25±0.06	3
30	A20B21	2.25±0.03	3
31	A14B6	2.24±0.10	4
32	A4B9	2.20±0.04	3
33	A13B12	2.19±0.03	4
34	A19B12	2.16±0.06	4
35	A12B3	2.16±0.15	4
36	A16B10	2.16±0.04	3
37	A13B3	2.16±0.12	3
38	A11B12	2.14±0.07	4
39	A7B2	2.14±0.13	4
40	A4B12	2.11±0.05	4
41	A19B3	2.09±0.10	4
42	A12B10	2.07±0.07	4
43	A14B14	2.02±0.12	3
44	A21B2	2.00±0.10	3
45	A13B2	2.00±0.05	4
46	A20B2	1.97±0.05	4
47	A9B8	1.96±0.07	3
48	A16B5	1.96±0.08	4
49	A14B10	1.94±0.05	4
50	A10B3	1.94±0.06	4
51	A8B8	1.94±0.04	4
52	A14B5	1.93±0.05	4
53	A15B19	1.93±0.10	4

54	A4B2	1.93±0.06	4
55	A14B2	1.92±0.12	4
56	A4B20	1.92±0.05	3
57	A6B10	1.87±0.05	4
58	A7B10	1.84±0.09	4
59	A21B10	1.81±0.08	4
60	A21B11	1.75±0.03	4
61	A9B10	1.60±0.02	4

300 Bold letters represented the strains selected for fruiting body production.

301 **Table 4. Comparison of the production yields and mycelial colonization times of fruiting**

302 **bodies**

Strains	Weight of fruiting body (g)	Biological efficiency (%)	Mycelial colonization time (days)
HS5	134.57±5.45 b ^a	40.78±1.65 b	30.82±0.98 a
HS4	91.43±5.24 cd	27.71±1.59 cd	24.72±1.01 g
A10B4	165.91±12.56 a	50.28±3.81 a	25.18±1.33 efg
A14B4	161.87±10.03 a	49.05±3.04 a	26.09±1.70 def
A8B6	123.20±12.84 b	37.33±3.89 b	26.36±1.69 cde
A7B6	102.67±17.46 c	31.11±5.27 c	29.82±1.08 a
A10B5	91.60±10.32 cd	27.76±3.13 cd	24.36±1.03 g
A1B11	74.70±6.59 d	22.64±2.00 d	26.91±0.94 bcd
A1B6	72.57±12.73 de	21.99±3.86 de	27.64±1.12 b
A1B17	54.47±8.43 ef	16.51±2.55 ef	27.27±1.62 cde
A1B12	54.33±14.33 ef	16.46±4.34 ef	27.36±1.43 bc
A1B18	47.33±3.79 f	14.34±1.15 f	25.00±1.18 fg

303 ^a Means in each column followed by the same letters are not significantly different at $P < 0.05$

304 according to Duncan's multiple range tests.

305

306 **Figure legends**

307 **Figure 1 Decolorization of HS4 and HS5 monokaryons on PDA plates containing RBBR.**

308 The RBBR fading degree was scored as 1 (+), 2 (++), 3 (+++), or 4 (++++). A, B, C, and D show
309 RBBR fading degrees of 1, 2, 3, and 4, respectively.

310 **Figure 2 Morphological characteristics of HS4, HS5, and crossbred strain fruiting bodies.**

311 **Figure 3 Amplification products obtained by PCR using ISSR primers and DNA from HS4,**
312 **HS5, and A10B4.** M, marker; line 1, HS4; line 2, HS5; line 3, A10B4. The arrows indicate the
313 different fragments.

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316

317

Fig 1.

Fig 2.

Fig 3.