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Development of improved strains of *Pleurotus ostreatus* with a shorter harvesting period and a higher yield through hybridization

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Abstract

Pleurotus ostreatus, a mushroom of significant commercial importance, is extensively cultivated worldwide. The demand for enhanced mushroom strains with increased yield is on the rise. This study aimed to enhance mushroom strains using the dual-culture hybridization method. Out of thirty-four combinations of single-spore cultures, ten successfully formed hybrids, as confirmed by the presence of clamp connections. Both hybrids and parent strains were assessed for their performance in spawn running, pinhead formation, yield, and biological efficiency. Compared to the parent strains, the time required for the spawn run and the initiation of pinhead formation in hybrids were reduced. The hybrid strain KAL5×A9.1 displayed the highest performance, achieving a biological efficiency (BE) of 79.61%, surpassing other hybrids and the parent strains. The color of the hybrids fell between those of the parents. Additionally, the hybrids exhibited greater stipe length and pileus diameter compared to the parental strains.

Keywords – Biological efficiency – clamp connections – dual-culture – hybrids

Introduction

Mushrooms have gained significant importance due to their nutritional and medicinal benefits and are cultivated worldwide (Chakravarty 2011, Dong et al. 2022). The consumer demand for mushrooms has increased in recent years, particularly for specialized mushrooms such as *Pleurotus* spp. The genus *Pleurotus* is a commercially important edible fungus commonly referred to as oyster mushroom (Barh et al. 2019). They are high in proteins, minerals, vitamins, amino acids, and fibers with less caloric value (Waktola & Temesgen 2018). These mushrooms also contain functional bioactive molecules (Kumar et al. 2021, Raman et al. 2021). They are the most cultivated mushrooms in Nepal, accounting for 86% of the total production (Raut 2019). These fungi are eco-friendly and can grow on a wide range of substrates. They are also utilized for the bioconversion of agricultural, industrial, and lignocellulosic wastes into enzymes and other chemicals for industrial and medical purposes (Kumla et al. 2020).

Mushrooms are primarily grown to achieve higher production and productivity, along with superior quality (Chakravarty 2011, Sharma et al. 2017). However, cultivated mushrooms face issues such as strain degeneration, loss of genetic diversity, and low yield due to prolonged cropping cycles (Jyothi & Thara 2021, Wang et al. 2012). To address these challenges, there is a need for the development of improved strains with high production and better-quality characteristics to meet both

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the grower and consumer demands (Baral et al. 2018, Kumara & Edirimanna 2009). Simple approaches like selection and hybridization can be easily applied to improve the characters by combining desirable traits from diverse strains and developing variation, i.e., hybrids that have the potential for industrial-scale use (Barh et al. 2019, Jyothi & Thara 2020).

Dikaryotization is one of the simple approaches used for hybridization in which a homokaryon is converted into a dikaryon by fusing with a compatible homokaryon or by merging a monokaryon with another dikaryotic mycelium. It is an important technique in strain improvement for introducing genetic recombination and producing hybrids with rapid colonization capacity, resulting in early maturation, good shape, size, and color of the fruiting body (Buswell et al. 1993, Larraya et al. 2003). Despite the popularity of mushrooms in Nepal for both food and medicinal purposes, no research has been conducted on the development of improved mushroom strains with better quality and productivity traits. Hence, the objective of this study is to create an improved strain of *Pleurotus* spp. by crossbreeding within *P. ostreatus*, aiming for better growth performance and an earlier flushing period.

Materials & Methods

Collection of mushroom samples

Two types of fresh *P. ostreatus* (KAL and A9) strains were collected from the Mushroom Seed Nepal and Research Center farm. The mushrooms were collected in sterilized plastic bags and taken to the research laboratory, Mushroom Seed Nepal and Research Center, Bhaktapur, for further experiments.

Preparation of spore print and monosporous cultures

The mature pilei of fruiting bodies were placed flat with gills down on the inside of sterile petri plates and incubated for 4 hours without any disturbance to get spore prints (Petersen & Ridley 1996). The spore print was suspended in 1 mL of sterile distilled water and serially diluted under sterile conditions. The spread plate method was used to obtain monosporous cultures. 0.1 mL of spore suspension was plated on Potato Dextrose Agar (PDA) and incubated at 25 °C for germination. After incubation, individual colonies were transferred to the PDA plates and incubated at 25 °C for seven days. Then, the monokaryotic mycelium was microscopically confirmed by the absence of mycelial clamp connections and maintained on PDA slants at 4 °C for storage (Bahukhandi & Sharma 2002).

Hybridization of monosporous cultures

Hybridization of monosporous isolates was performed using a dual culture technique. The two different monokaryons were inoculated 5 mm apart on a PDA plate and incubated at 25 °C for seven days. Three replicate plates were prepared for each combination. The fused mycelia showing profuse hyphal development at the point of contact was cut, transferred to new PDA plates, and examined for clamp connections under the microscope after incubation for seven days. Only the hybridized strains, with successful clamp connection, and parents were then further processed for investigation (Bahukhandi & Sharma 2002, Gordon & Petersen 1992).

Production of Spawns

Spawn of *P. ostreatus* parents and their hybrids were prepared. First, wheat was boiled for two hours, rinsed, and then left to cool to 25 °C. A mixture of cooled wheat grains with 1% calcium carbonate and 1% gypsum was then filled into sterile plastic bags (200 g), sealed, and autoclaved for 1 hour at 121 °C. The bags were then inoculated with fresh mycelium and incubated at 25 °C until the grains were entirely covered by mycelia (Farimani & Farsi 2023).

Fruit-body production

The wheat straw was dried, crushed, and soaked for 24 hours. The straw was boiled for a few hours, then dried under aseptic conditions, and packed in sterile bags (2 kg/bag). The bags containing

straw were inoculated with 200 g of mature spawn, followed by shaking to distribute the spawn evenly all over the bag. The bags were then incubated for spawn run under complete darkness at room temperature (25 ± 4 °C). When the mycelium reached the bottom, the bag was cut open and kept in the cultivation room to initiate fruiting. The bags were watered regularly to maintain a humidity level of 75–85%. The mushrooms were harvested at the adult stage when the pilei were fully developed (Farimani & Farsi 2023).

The total weight of the fruiting bodies was recorded during each harvest using a weighing scale. Parameters such as spawn running, pinhead formation, fruiting body yield, and biological efficiency were measured. Productivity was expressed as biological efficiency (BE) ([fresh weight of mushrooms harvested/dry weight of substrate used] × 100) (Jyothi & Thara 2020). Parent and hybrid sporophores were examined for size, color, shape, margin, and texture of the pileus.

Statistical analyses

The data were evaluated using One-way Analysis of Variance (ANOVA) to assess the significance of individual differences at $p<0.05$ level. Duncan's multiple range test was used to compare significant means. All statistical analyses were carried out using the SPSS statistical software package (Version 23) and Microsoft Excel 2013.

Results and Discussions

The monosporous cultures from A9 and KAL successfully hybridized (Figure 1a) and produced heterokaryotic mycelium in 10 combinations out of 34, and the success rate was 29.4%. Success was confirmed by the presence of clamp connections on the hyphae of the hybrids, showing sexual compatibility (Figure 1b). The hybridized area could represent the degree of compatibility between the two strains. A previous study reported a high rate (92%) of mating from *P. ostreatus* (Kumara & Edirimanna 2009). The observed rate of mating could be attributed to morphological characteristics from the species of the same genus (Rosnina 2007).

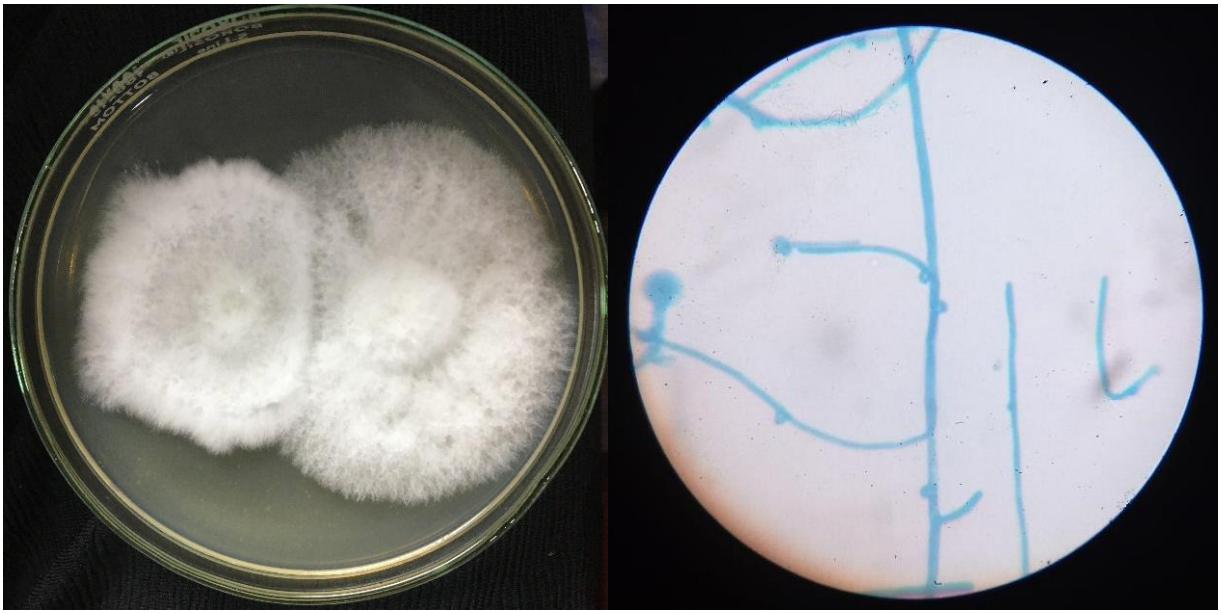

Fig. 1 – Compatibility mating by dual culture technique of KAL5 × A9.1. a Compatible mating reaction. b Clamp connection in hybrid.

The spawn running, pinhead formation, and weight of fruiting bodies of hybrids and parent strains are presented in Table 1. The hybrids completed spawn run within 27 to 32 days, which is shorter in comparison to the 33–37 days taken by the parent strains. However, these findings of the spawn run did not agree with the results of Kumara & Edirimanna (2009), who stated that *P. ostreatus*

completed the spawn run in 24 to 26 days. The variation in the number of days required for spawn colonization depends on the fungus strain, substrate type, and growth medium (Miles & Chang 2004).

The pinheads formed at different times in hybrid strains. Specifically, KAL5 × A9.1 and KAL3 × A9.3 took fewer days (11–14 days) to form primordia compared to other hybrid strains and parents. However, other studies showed that the days taken for pinhead formation were 21–23 days in *P. ostreatus* (Sitaula et al. 2018). Early pinhead production could be interpreted as an indicator of faster fructification potential (Gaitán-Hernández & Salmones 2008). The hybrids KAL5 × A9.1 and A9.2 × A9.5 took the shortest time of 26–32 days to harvest the first fruiting bodies, whereas the parents took a longer time (33–41 days). The spawn running, pinhead formation, and fruiting bodies production are three essential stages in mushroom cultivation that need proper temperature and humidity (Hoa & Wang 2015).

In our study, the yield of some hybrid strains was remarkably higher than that of the parents, whereas some of the hybrids yielded less compared to the parents. Specifically, hybrid KAL5 × A9.1 and A9.2 × A9.5 yielded the highest crop in the three flushes, with yields of 796.14 ± 0.02 g and 623.2 ± 0.03 g, respectively. The yield of parent strains A9 and KAL was 469.2 ± 0.04 g and 612 ± 0.03 g, respectively, which were lower than some of the hybrid strains. Biological efficiency refers to a strain's ability to convert the substrate into a more useful form (Kumara & Edirimanna 2009). The BE of KAL5 × A9.1 was higher (79.61%) compared to the parent and other hybrid strains. This result is consistent with the study by Jyothi & Thara (2020). The lowest biological efficiency values were observed in A9.1 × A9.7 and A9.7 × A9.8 (19.72% and 21.96%, respectively).

Table 1 Spawn run period and biological efficiency of hybrid and parental *P. ostreatus.*

Parents and hybrids	Incubation time (Days taken for)			Yield for three flushes (g)	Biological efficiency
	Complete spawn run	Pinhead formation	First harvest		
Parents					
A9	34 – 37	20 – 25	36 – 41	469.2 ± 0.04^{bcd}	46.92
KAL	33 – 35	17 – 22	33 – 36	612 ± 0.03^{d}	61.2
Hybrids					
KAL3 × 5	29 – 31	13 – 16	27 – 31	423.8 ± 0.02^{bc}	42.38
KAL3 × A9.3	28 – 30	11 – 14	27 – 35	410 ± 0.01^{bc}	41
KAL5 × A9.1	29 – 32	11 – 14	26 – 32	796.14 ± 0.02^{e}	79.61
KAL5 × A9.8	27 – 29	17 – 19	31 – 36	580.2 ± 0.04^{cd}	58.02
KAL5 × 8	27 – 30	12 – 15	27 – 32	463.2 ± 0.02^{bc}	46.32
A9.1 × A9.7	28 – 32	18 – 20	31 – 37	197.2 ± 0.01^{a}	19.72
A9.2 × A9.3	29 – 30	14 – 17	27 – 34	288.6 ± 0.02^{ab}	28.86
A9.2 × A9.5	29 – 31	13 – 19	26 – 32	623.2 ± 0.03^{d}	62.32
A9.7 × A9.8	28 – 30	16 – 19	32 – 36	219.6 ± 0.01^{a}	21.96
A9.7 × KAL8	30 – 32	13 – 17	27 – 33	502 ± 0.01^{cd}	50.2

Values mean ± SD of five replications. Values with a similar letter (s) are not significantly different from one another ($p > 0.05$) using DMRT.

The pileus and stalks of hybrid strains exhibited a coloration range of white, grayish white, and gray, representing an intermediate between that of their parent strains. This might be due to the phenotypic characters of one of the parent strains being expressed in hybrid strains (Mallick & Sikdar 2014). The pileus margins of hybrids were mostly undulating in appearance, while few showed uniformity. Most of the hybrids were funnel-shaped, differing from their oyster-like parents. The shape of the hybrids was not as consistent or appealing compared to the parents (Fig. 2).

Fig. 2 – Morphological characteristics of both parental strain and hybrid sporophores. a Parent strain A9. b Parent strain KAL. c Hybrid A9.2 × A9.3. d Hybrid KAL3 × A9.3. e Hybrid KAL5 × A9.8. f Hybrid A9.1 × A9.7. g Hybrid A9.7 × KAL8. h Hybrid KAL3 × 5. i Hybrid A9.7 × A9.8. j Hybrid A9.2 × A9.5. k Hybrid KAL5 × 8. l Hybrid KAL5 × A9.1.

In our study, the parental strains contributed to the larger pileus diameter and stipe length in the hybrids. The hybrid A9.1 x A9.7 produced the largest fruiting bodies with a pileus diameter of 17 ± 0.08 cm, whereas the longest stipe length was found in the strain KAL5 x 8 (11.1 ± 0.14 cm), followed by KAL5 x A9.8 (11 ± 0.16 cm) (Table 2).

Table 2 Comparison of morphological characteristics and traits of hybrids with parental strains.

Parents and hybrids	Stipe length (cm)	Pileus diameter (cm)	Color of pileus	Margin/shape of pileus	Sporophores texture
Parents					
A9	6.5 ± 0.08^{c}	6.5 ± 0.08^{b}	Light greyish white	Undulating, Oyster-shaped	Fleshy
KAL	5.2 ± 0.14^{b}	11 ± 0.63^{f}	Grey white	Uniform, Oyster-shaped	Fleshy
Hybrids					
KAL3 x 5	9 ± 0.24^{f}	14.1 ± 0.08^{h}	White	Undulating, Funnel-shaped	Fleshy
KAL3 x A9.3	9 ± 0.08^{f}	15 ± 0.16^{i}	Grey	Undulating, Oyster-, Funnel-shaped	Fleshy
KAL5 x A9.1	6.5 ± 0.08^{c}	11 ± 0.24^{f}	Whitish	Undulating, Funnel-shaped	Fleshy
KAL5 x A9.8	11 ± 0.16^{g}	8.2 ± 0.28^{d}	Grey	Undulating, Funnel-shaped	Fleshy
KAL5 x 8	11.1 ± 0.14^{g}	6.2 ± 0.16^{b}	White	Undulating, Funnel-shaped	Fleshy
A9.1 x A9.7	6.7 ± 0.16^{c}	17 ± 0.08^{j}	White	Undulating, Funnel-shaped	Fleshy
A9.2 x A9.3	7.1 ± 0.08^{d}	9 ± 0.24^{e}	Grey	Uniform, Oyster-shaped	Fleshy
A9.2 x A9.5	8 ± 0.16^{e}	12.1 ± 0.14^{g}	Grey, white	Undulating, Funnel-shaped	Fleshy
A9.7 x A9.8	4.1 ± 0.08^{c}	7.6 ± 0.22^{c}	Grey	Undulating, Oyster-shaped	Fleshy
A9.7 x KAL8	6.5 ± 0.16^{c}	5.1 ± 0.22^{a}	Light greyish white	Uniform, Funnel-shaped	Fleshy

Values mean ± SD of 3 sporocarps. Values with a similar letter (s) are not significantly different from one another ($p > 0.05$) using DMRT.

Conclusion

The obtained hybrids were found to be superior to their parents, with a quick spawn run period, high yield, maximum biological efficiency, and improved pileus size, favoring consumer preference.

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Competing interests

The authors declare no conflicts of interest concerning the research, authorship, and publication of this article.

Authors' contributions

KR: conducted laboratory experiments, analyzed the data, and reviewed the manuscript. MS: conducted laboratory experiments, analyzed the data, drafted, and revised the manuscript. AB: designed the study and conducted laboratory experiments. JK: supervised and reviewed the manuscript. All authors read and approved the final manuscript.

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