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Morphological characterization of *Lentinulla edodes* hybrid strains obtained by intraspecific mating

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ABSTRACT

Mushroom production started in 1960s and expended rapidly in the 1990s but mushroom as a vegetable is yet to find regular place in the Indian agri-food landscape. Among various cultivated species of mushrooms, shiitake mushroom has a good demand among consumers particularly in northern India due to its numerous beneficial properties like its taste and medicinal value. Presently, the China and Japan are the bulk producers of this prized mushroom. The current study attempted to generate improved strains of *L. edodes* by using intraspecific hybridization. Each of the five parent monokaryotic mycelia (LE-1501, LE-1502, LE-1503, LE-1504 and LE-1505) were mated with the remaining four monokaryons resulting in a total 10 mating. Out of 10, only 5 compatible mating strains (Hybrid Spore Pair 1-2, 1-4, 2-3, 3-4 and 4-5) were generated in which clamp connections were confirmed. The significant variations among the newly generated hybrids were identified by morphological visualization. Among five, only three hybrids are totally different from the parents. The strain HSP 1-2 expressed co-dominance by producing distorted, creamy white and honey brown pileus having white scars observed on some of the fruits with stumpy stipe. While hemispherical, light brown cap with flat, thin and irregular margin was the characteristics of HSP 2-3 and the strain HSP 4-5 produced distorted spherical, dull white cap with white scars uniformly distributed from outside. This research involved a successful initial attempt to use a broad range of analyses and breeding to generate superior hybrids of *L. edodes*.

Key words: *Lentinulla edodes, Hybridization, Morphological characterization, Mating*

Introduction

India has massive amount of lignocellulosic waste and cultivation of edible fungi is one of the most captivating methods of their recycling. It is not only helping in the management of horticultural and agricultural wastes, but also supports in the production of nutrient-rich vegetables and generation of additional employment. Mushrooms are also known as 'white vegetables' or 'boneless vegetarian meat', contain ample amounts of vitamins, proteins and fiber and have certain medicinal properties (Standish *et al*., 2008; Xu and Beelman, 2015; Girma and Tasisa, 2018; Binjola *et al*., 2019). Mushroom contains 20-35% protein (on dry weight basis) which is higher than exotic fruits and vegetables (Barros *et al*., 2008; Ho *et al*., 2020). Compared to other vegetables, per capita consumption of mushrooms in India is less than 100 grams per year, which is very low as compared to China 10 kg/person/year. The per capita consumption of mushroom in China is even higher than in US and many European coun-

tries where it is around 3kg/person/year (Wakchaure, 2011). In 2018-2019, global mushroom production was 43 MT with *Lentinula edodes* (Shiitake) contributing 26%, *Auricularia* spp. (Wood ear) 21%, *Pleurotus ostreatus* (Oyster) 16%, *Agaricus bisporus* (Button) 11%, *Flammulina velutipes* (Enoki) 7%, *Pleurotus eryngii* (king oyster) 5%, *Volvariella volvacea* (paddy straw mushroom) 1% and others 13%. At present, the total mushroom production in India is approximately 0.15 MT, from 2010-2018, the mushroom industry in India has registered an average growth rate of 4.3% per annum (Gautam, 2020). However, cultivation technologies of many exotic mushrooms were standardized; but the commercial markets are still dominated by *Volvariella volvacea, Pleurotus* spp. and *Agaricus bisporus*. These three mushrooms are contributing about 96% of total mushroom produced in India (Gautam, 2020). *Lentinula edodes* are now getting significant importance due to their nutritional and medicinal value. In order to generate high performance strains of mushrooms, a range of breeding methods have been established (Chang, 2006).

Improved strains not only increase the mushrooms quality, but also reduce cultivation costs. They can also increase farmers' income in the short term (Marshall and Nair, 2009; Avin *et al*., 2012). Newly generated hybrid strains of mushroom have been investigated using various markers, such as morphological (Adebayo *et al.,* 2013), culture assessments (Gharehaghaji *et al*. 2007), mating tests (Shnyreva *et al.,* 2012) and fruiting trials (Ramirez *et al*., 2011).

Materials and Methods

Preparation of single spore isolates

In the present study, five strains of *L. edodes* viz LE-1501, LE-1502, LE-1503, LE-1504 and LE-1505 were used as parents. Basidiospores were obtained from spore print of mature sporophores. Then, the petri plates containing potato dextrose agar (PDA) media was inoculated with basidiospores and the petri plates were incubated up to 4-7 days at 24±2 ^{0}C for spore germination.

Preparation of monokaryotic isolates

Single spore isolates of each parental strain were isolated and cultured on PDA media. The mycelium cultures were validated as a monokaryon by the absence of clamp connections on the hyphae while examination under a microscope. Monokaryon cultures of parental strains were selected for mating studies.

Mating between monokaryotic isolates of *L. edodes*

Each of the five monokaryotic mycelia (LE-1501, LE-1502, LE-1503, LE-1504 and LE-1505) were mated with the remaining four monokaryons resulting in a total 10 mating. In all the combinations, inoculum plug of 0.5 cm diameter was placed in juxtaposition at the center of a 90 mm diameter petri dish containing PDA, each monokaryotic isolates were placed in pairs. The two strains were located approximately 1 cm apart from each other on a petri dish. Then, they were left till resultant colonies overgrew the space between the inoculum and developed a contact zone, usually after 7 to 10 days.

Spawn production and Cultivation of the hybrid dikaryons.

Wheat grain was used as substrate for spawn preparation and there after processed grains were filled in poly propylene bag. Each bag contained half or one kg of wheat grain substrates after that these bags was sterilized in autoclave at 15 psi pressure for 2 hours. Bags were aseptically inoculated with 25-30 g of mother spawn in laminar air flow and incubated at 24±2 ^{0}C. The inoculated bags were frequently examined for any types of contamination and those bag exhibiting contaminations discarded immediately, while those showing whitish mycelial growth covering all the grains were used for experimentation.

For the cultivation, the spawn were inoculated onto solid substrate consisting sterilized sawdust in a wide-mouth polypropylene bag under aseptic condition. Then the spawned bags were kept into crop house for further development.

Results

Development of hybrid strains via mating process
Out of 10, only five compatible mating strains (Hybrid Spore Pair 1-2, 1-4, 2-3, 3-4 and 4-5) were generated in which clamp connections were confirmed (presented in Table 1). Then a small piece of about 0.5 cm of mycelium were cut off from the junction zone of encounter between the two colony cultures, then sub-cultured to a new PDA plate and incubated at 24±2 ^{0}C for 10 to 14 days. After purification

these strains were further used for spawn production and cultivation.

Table 1. Mating between monokaryon isolates of *Lentinula edodes*

	1	**2**	**3**	**4**	**5**
1					
2	O				
3	X	O			
4	O	X	O		
5	**X**	**X**	**X**	**O**	

Mating pairs were carried out in triplicate. "O" signifies full compatibility in which a fertile and non-restricted dikaryon with true clamp connections is formed. While "**X**" signifies incompatility, in which an infertile, principally monokaryotic heteyrokaryon without clamps is formed. The number 1, 2, 3, 4, and 5 designated as LE-1501, LE-1502, LE-1503, LE-1504 and LE-1505, respectively.

Morphological characterization

The newly generated intra-specific hybrids of *Lentinula edodes* were cultured on a suitable substrate in order to identify different and superior hybrids. The hybrid strains were cultured by three replications, with each replication containing five to ten individual bags to test productivity and stability of the selected hybrid strains. The following measurements were carried out on the generation: the size of the pileus and stipe of the harvested basidiocarp; the shape of basidiocarps (normal, malformed); the shape, colour, turgidity and thickness of pilei; the length, thickness and turgidity of stipes and it is described in the Table 2.

The Figure 1 exhibited morphological characteristics of the parent and as well as hybrid strains of *L. edodes*. All the parental strains were circular in shape but the strain LE-1501 having white scales scattered throughout the pileus as well as circular white ring of scales was also a prominent features of this strain. The strain LE-1502 was dark brown colour with multiple cracks on pileus but LE-1503 and LE-1505 wwere also dark brown with white scale scattered on some fruits. The strain LE-1504 was dark brown with white appressed structure appear on the fruits from periphery to center. Among five hybrids, the strain HSP 1-2 expressed co-dominance by producing distorted, creamy white and honey brown pileus having white scars observed on some of the fruits with stumpy stipe. The spherical, blackish brown to light brown from center to outside with wavy margin pileus were observed on HSP 1-4. Hemispherical, brown cap with flat, thin and irregular margin was the characteristics of HSP 2-3. Strain HSP 3-4 expressed spherical, flat pileus with white scars uniformly distributed throughout the cap and HSP 4-5 produced distorted spherical, dull white cap with white scars uniformly distributed from outside.

Discussion

It is a goal of mushroom breeders to produce hybrid strains whose fruiting bodies has qualities equivalent to commercially accepted in yield, flavor, fruiting time, texture and nutritive value Chang and

Table 2. Characterization of hybrid strains produced after mating.

Strains	Stipe length/ pileus diameter (cm)		Characteristics	Texture/ Aroma
HSP 1-2	5.46 ± 0.470	11.50 ± 0.173	Distorted, creamy white and honey brown cap with stumpy stipe, white scars observed on some of the fruits.	Papery/ less earthy smell.
HSP 1-4	5.93 ± 0.186	12.70 ± 0.379	Spherical, blackish brown to light brown from center to outside with wavy margin.	Papery/ strong smell.
HSP 2-3	5.83 ± 0.219	13.30 ± 0.208	Hemispherical, brown cap with flat, thin and irregular margin.	Fleshy/ decrease smell.
HSP 3-4	5.93 ± 0.145	11.83 ± 0.240	Spherical, flat pileus with white scars uniformly distributed throughout the cap from outside.	Fleshy/ strong smell.
HSP 4-5	6.20 ± 0.346	12.76 ± 0.176	Distorted spherical, dull white cap with white scars uniformly distributed from outside.	Fleshy/ decrease smell.

*Each value is expressed as mean ± standard error (n = 3)

Miles (2004). The irregular morphologies generated by the breeding will be very useful resource to understand the molecular mechanism (Shimomura *et al.*, 2007). In this scientific study, we have demonstrated the variation in morphological characteristics of the parent and hybrid strains of *L. edodes.* There is no report yet on breeding in L. *edodes* so far (Akhtar *et al*., 2022), but similar research has been done on different mushroom by various scientists and here are some examples. Toyomasu and Mori (1987) worked on protoplast fusion of *Pleurotus species* resulting in some of the fusion produced were morphologically abnormal. Dhitaphichit and Pornsuriya (2005) observed that out of 412 isolates, 2 isolates possess clamp connection and both the fusants showed morphological variations while working on protoplast fusion of *P. ostreatus* and *P. djamor.* Jaswal *et al*. (2014) worked on *P.* var. *florida* and *P. sajor-caju* and developed five hybrid dikaryons which was different from parents in terms of quality, enzyme profile, relatedness, linear growth rate, etc. Rosnina *et al.* (2016) were crossed ten monokaryon cultures of the parental strains of *P. citrinopileatus* and *P. pulmonarius* in all combinations to obtain hybrids and few of them were found good in color and yield.

Conclusion

This work displayed that some hybrid strains generated after mating of parental monokaryons of *L. edodes* exceed the parental sporophore characteristics and this was worthy of further investigation. The various irregular fruiting bodies generated by hybridization will be useful resources for the experimentation on molecular studies of mushroom. In current situation, the research of bioactive components in wild and edible mushroom is yet deficient. There are various potential characteristics of mushrooms with nutraceutical and health benefits, which deserve future investigations.

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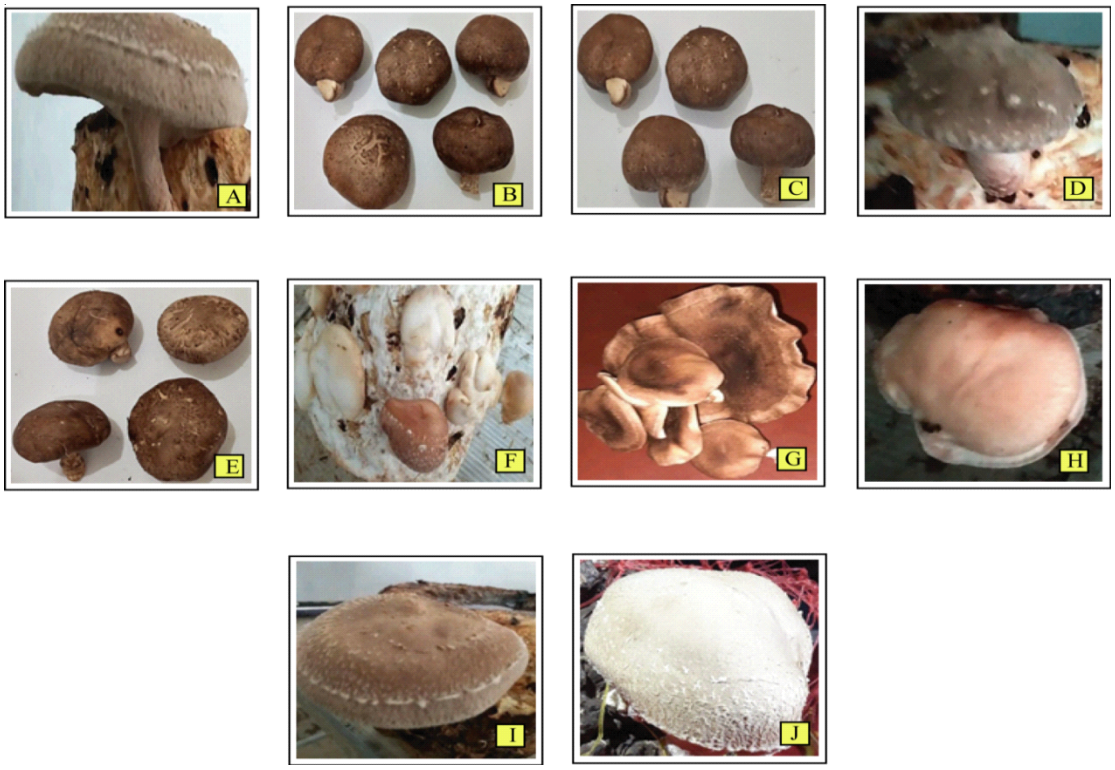

Fig. 1. Morphologies of parent and hybrid fruiting bodies. A-E, Parental strains LE-1501, LE-1502, LE-1503, LE-1504 and LE-1505 respectively; Hybrid strains F, HSP 1-2; G, HSP 1-4; H, HSP 2-3; I, HSP 3-4; and J, HSP 4-5.
*HSP indicate Hybrid Spore Pair

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