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MATING TYPE ANANLYSIS OF OYSTER MUSHROOM (*PLEUROTUS FLABELLATUS*) USING SINGLE BASIDIOSPORES FOR STRAIN IMPROVEMENT

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ABSTRACT
The single spores were isolated using a new technique that is less prone to contamination and more efficient than the common techniques used by earlier workers. Mating types of all the isolates within the species were identified on the basis of hyphal fusion via anastomosis with the tester strains. Two compatible pairs of isolates with well prominent tuft in the contact zone were selected for dikaryon isolation. The dikaryotic isolates with their replicates were evaluated for spawn run period, pin head initiation, total no. of sporophore, yield and biological efficiency. The minimum days were required for spawn run were observed in strain of P flab (7 x 35) 6 (11.33 days) and strain of P flab (35 x 120) 5 (12 days). The minimum days required for pin head initiation were observed in strain of P flab (7 x 35) 1 (4 days) and strain of P flab (35 x 120) 15 (4.33 days). Total no. of sporophores were produced highest in strain of P flab (7 x 35) 9 (24.44) and strain of P flab (35 x 120) 5 (26.11). The higher yield and higher biological efficiency were reported in strain of P flab (7 x 35) 1 (401.67 gm, 80.33%) and strain of P flab (35 x 120) 2 (447 gm, 89.41%) of *P. flabellatus*.

INTRODUCTION

Mushrooms are regarded as highly nutritious food containing large amount of proteins. Mushrooms are also important foreign exchange earners. Mushrooms have been recommended as food item contributing significantly to the protein nutrition of the developing countries like India (FAO), which depend mainly on the cereal diets. (Sharma *et al*., 2010). The cultivation of edible mushrooms is a biotechnological process that uses various residues to produce food of high nutritional value which can be a solution to problems of global importance, such as the lack of protein in developing countries and the possibility of environmental management (Sharma *et al*., 2013). Mushroom in general and *Pleurotus* in particular are an important source of nutrition particularly for the people on cereal-based diet. Edible species of mushroom are low in calories, fats, sodium, carbohydrates and cholesterol, whereas, rich in proteins, minerals, vitamins and fibers (Nasim *et al*., 2001). Besides nutritional importance genus *Pleurotus* is also known for its medicinal value (Kothe 2001), paper pulp bleeching, cosmetics and industrial use (Kyung-Ho Ma *et al*., 2009; Sigoillot *et al*., 2005). However, *Pleurotus* is ranked second to the button mushroom (Kues and Liu 2000), but low yield level, inconsistency in flush appearance, texture, color and taste affects its adoption as the favorite mushroom for cultivation. The need of the hour is not only to explore other new species but also to improve the existing species through various breeding techniques for higher yield, better quality, texture, color and taste to meet the rising demands of the increasing population. The breeding program for mushrooms in India has been limited to the import of strains from advanced countries for direct introduction, but the procurement and introduction of the existing commercial strains from abroad is a short-term strategy of mushroom breeding. Besides introduction, selection has also been reported in many cases using tissue culture (Kligman 1943), multispore culture (Fritsche 1981) and single spore isolation (Kumar 1987; Bisko and Kholodony 1989). Although these processes are very important for revitalizing the strains but hybridization based on the crossing of non-fertile homokaryotic lines offer better prospects for genetic improvement. For developing the mushroom hybrids possessing better traits, primary step is the generation and characterization of the pure genetic material i.e., single basidiospores, the true meiotic products. In the present study, we isolated single basidiospores from *P. flabellatus*. Monokaryotic isolates helped us to further analyze the mating types and compatibility status of the isolates and derivation of dikaryons from the compatible mating pairs

MATERIALS AND METHODS

Isolation of single basidiospores

Mature, healthy and fresh mushroom bodies of *P. flabellatus* (P flab) collected from Department of Plant Pathology and Mushroom Division of IGKV, Raipur, India, were used for the isolation of single basidiospores. Single basidiospore isolation was done following the technique developed by Kotasthane (2010).

Morphological characterization of single basidiospores

Cultural characters of single basidiospores derived from the mushroom fruiting bodies were evaluated. All the isolates were grown on sterilized petri-plates which contain 20-25mL of PDA. A disc of approximately 5 mm diameter from the PDA slants was transferred aseptically at the center of each petri-dish and incubated at 27 ± 1ºC. The colour of the colony was determined. Observations on radial growth, colour of the colony, appearances were recorded after completion of growth in any one of the isolate. Growth rate of the fungus measured using radial growth measuring scale.

Identification of single basidiospore mating types

Mating types of the single spore isolates were analyzed using two-point inoculation technique (Kotasthane 2003). Small block of the fungus cut from the growing culture of the two different isolates were transferred about 3 cm apart from each other in a single fresh petri-plate (Fig. 1) poured with 20–25 ml sterilized PDA and incubated at 27 ± 1 ºC. All the combinations were observed regularly. The positive or compatible mating types were recognized by the presence of barrage or fluffy and vigorous mycelia at confrontation zone (Fig 1). We selected the compatible pair of isolates which was having well prominent interaction zone as tester strains. Mating types of the single basidiospore cultures used as testers were designated as AxBx and AyBy. Mating type of all the other isolates were determined by mating each isolate with both the tester strains.

Dikaryon isolation and evaluation for yield and biological efficacy

Among compatible interactions we selected some well prominent interaction zone for isolating the dikaryons by cutting and transferring the mycelia to fresh PDA plates. Mother spawn of *P. flabellatus* were prepared on grains. Wheat grains were used as substrate. Clean, healthy and bold grains of wheat were taken while broken and undersized grains were removed. These were submerged in tap water for overnight, on next day, water was decanted and grains were boiled for 10-15 minutes till they became soft while seed coat remained intact. Thereafter, excess water was run off and evenly spread on muslin cloth for cooling. The cooled grains were then mixed with calcium carbonate (0.5%) and copper sulphate (2%) on wet weight basis to avoid clumping of grains and maintenance of pH. In empty glucose bottles or 250 ml conical flasks, these processed grains were filled up to half capacity and plugged with non-absorbent cotton then sterilized in an autoclave at 15 lb PSI or 121.6 ºC for 2 hours. After sterilization, flasks were cooled and inoculated with a small bit of 5mm diameter of pure culture and incubated for 15 days at 25 ± 2ºC till the white mycelium covered all grain surfaces.

Mushroom bags were prepared for cultivating the developed spawn in the culture room. Wheat straw was chopped into pieces of 4–5 cm and dipped into the water (already mixed with 75 ppm Bavistin and 500 ppm formaldehyde for 14 h). Excess water was drained off and straw was air dried till the moisture content declined to 60–70%. Spawn was mixed with the substrate @ 4% on wet weight basis. 1.5 kg of spawned substrate was filled into the polythene bags (12''X 18''-150 guage) and nylon string was employed for tying the mouth of the bags. Perforations were made with the help of paper pin or nail to allow free passage of air within the bags. Each spawn was equally replicated in three different bags and watered regularly to maintain high humidity (70–85%) and low temperature (25–30 ºC). When the straw was fully covered with the milky mycelial growth, it was regarded as the period of complete spawn run. At this stage, the bags were cut open and the compact mass of aggregated straw termed as ''bed'' was ready for hanging on the wooden rack. Pinheads started appearing after 3–5 days of the bag removal. Mature sporophores were picked up just before the edges of the pileus begin to curl or fold downwards. Successive two or three flushes were harvested from the same bed at an interval of 9-10 days. Freshly harvested sporophores from each bag were counted and weighed immediately. Two or three flushes represented weight (g) per unit of dry straw substrate. Both the yield and biological efficacy were estimated from the collected data.

Statistical analysis

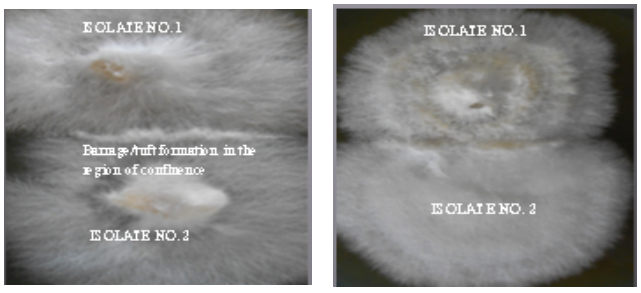
The experiment was conducted in a controlled randomized design (CRD). The treatment means were compared using critical difference (CD) at p = 0.05.

RESULTS AND DISCUSSION

Isolation of single basidiospore isolates

In order to select the single basidiospore isolates with better traits for developing inter-strain/intra-species hybrids, we isolated 50 single spore isolates from *P. flabellatus* (designated as P flab –P flab in the present study. A new approach developed by Kotasthane (2010) was used for isolating the

Magnified view of barrage/tuft formation in the region of confluence



Two point inoculation techniques in plates indicating the compatible mating pairs as confirmed by the barrage / tuft formation in the confluence region

Figure1: Two-point inoculation technique in plates for the identification of compatible mating pairs

basidiospores. The efficiency of this method is very high and in a single attempt as many as 60–80 basidiospore progenies can be obtained. The technique of basidiospore isolation is direct, simple, rapid and the chances of recovery of a true product of meiosis are quite high (Kotasthane and Singh 2000; Kotasthane 2003). Unlike earlier methods (Kumar and Munjal 1981; Perisemy and Nataranjan, 2003; Yadav *et al.*, 2003), this procedure of single spore isolation involves minimal use of water, no surface sterilization of the source material and also the spores being isolated can be clearly seen and easily lifted from the media.

Table 1: Mating type and compatibility status of single basidiospore isolates (derived from *P. flabellatus*) with the putative parental strains P flab 7 (AxBx) and P flab 35 (AyBy)

S. No.	P flab 7 (AxBx)	P flab 35 (AyBy)
1	P flab 35 (AyBy)	P flab 7 (AxBx)
2		P flab 49 (AxBx)
3		P flab 120 (AxBx)

P flab 7 was slow growing and P flab 35 was fast growing; both are compatible

Morphological characterization of basidiospore isolates

Cultural morphology varies greatly within isolates. Fifty single basidiospore monokaryotic isolates were grown on Potato

Table 2: Yield performance of selected strains of P flab (7x35) of *Pleurotus flabellatus* on wheat straw substrate

Yield g/500 gm dry wheat substrates Strains	Spawn run(DAI)	Pin head initiation (DAS)	Pileus diameter (cm)	Stipe length (cm)	Stipe diameter (cm)	No. of sporophore	Total Yield (gm)	B.E. (%)
P flab(7x35)1	12	4	7.85	3.36	0.84	17.67	401.67	80.33
P flab(7x35)2	12	5.67	6.16	2.5	0.63	17.89	378.33	75.66
P flab(7x35)5	12.67	5.33	5.71	2.15	0.71	15	383	76.66
P flab(7x35)6	11.33	5.33	6.64	2.66	0.69	11.33	305.33	61.06
P flab(7x35)9	14	5.33	7.61	3.39	0.85	24.44	388.33	77.66
P flab(7x35)10	12.67	4.33	4.7	2.26	0.65	19.22	317	63.4
P flab(7x35)11	12	6	5.26	2.91	0.76	14.67	281	56.2
P flab(7x35)13	14.67	4	5.64	2.94	0.82	19.22	389	77.8
P flab(7x35)14	12.67	4.67	5.45	2.42	0.68	10.78	213.33	42.66
P flab(7x35)15	11.33	6	7.34	3.45	0.83	20	344.33	68.86
P flab(7x35)16	12	5.33	7.14	3.19	1.1	19.22	387.67	77.53
P flab(7x35)18	13	5.67	6.25	2.55	0.7	12.11	249	49.8
P flab(7x35)19	12	4.67	5.32	2.83	0.81	13.89	224.67	44.93
P flab(7x35)22	12	5.33	6.42	2.73	0.89	16.67	315	63
P flab(7x35)23	12	5.33	4.98	2.73	0.75	17	280.67	56.13
Control	12.67	5	5.98	2.55	0.75	15.44	265	53
CD (P=0.05%)	1.78	1.22	1	0.50	0.23	5.11	25.92	
SE (m) ±	0.62	0.42	0.35	0.17	0.08	1.77	9.00	

Table 3: Yield performances of selected strains of P flab (35 x 120) of *Pleurotus flabellatus* on wheat straw substrate

Yield g/ 500 gm dry wheat substrate Isolate/strain	Spawn run(DAI)	Pin head initiation (DAS)	Pileus diameter (cm)	Stipe length (cm)	Stipe diameter (cm)	No. of sporophore	Total Yield	B.E. (%) (gm)
P flab(35x120)1	12.33	5	6	2.43	0.83	24.11	444	88.81
P flab(35x120)2	12.67	5.33	6.3	2.65	0.78	22.33	447	89.41
P flab(35x120)5	12	5.33	8.3	3.09	0.81	26.11	433.33	86.66
P flab(35x120)6	12	5.67	5.3	2.39	0.8	18.44	342	68.4
P flab(35x120)9	12.67	6.67	8.1	3.23	0.98	16.44	303.67	60.73
P flab(35x120)11	12.67	5.67	5.1	2.57	0.82	13.11	298.67	59.84
P flab(35x120)12	12	5	6.8	2.44	0.86	18.44	345	69
P flab(35x120)13	14.33	6.67	8.6	3.66	1.05	18.33	325.67	65.13
P flab(35x120)15	12. 33	4.33	5.5	2.13	0.75	14.22	315.33	63.06
P flab(35x120)17	12. 33	5.67	6.5	2.57	0.85	18.44	378.33	75.66
P flab(35x120)19	12. 33	5	5.7	2.47	0.89	17.56	318	63.61
P flab(35x120)20	15. 33	5	7.1	2.65	0.85	20.89	394.67	78.93
P flab(35x120)22	13. 33	4.67	5.6	2.23	0.75	19.33	322.33	64.46
P flab(35x120)23	12. 33	5	6.6	2.42	0.65	16.22	272.33	54.46
P flab(35x120)24	12	4.33	7.3	2.53	0.93	17.67	359.33	71.86
Control	14	6	6.1	2.54	0.79	14.33	298.33	59.66
CD (P=0.05%)	1.59	1.40	0.90	0.53	0.17	5.88	31.84	
SE (m) ±	0.55	0.49	0.31	0.18	0.06	2.04	11.05	

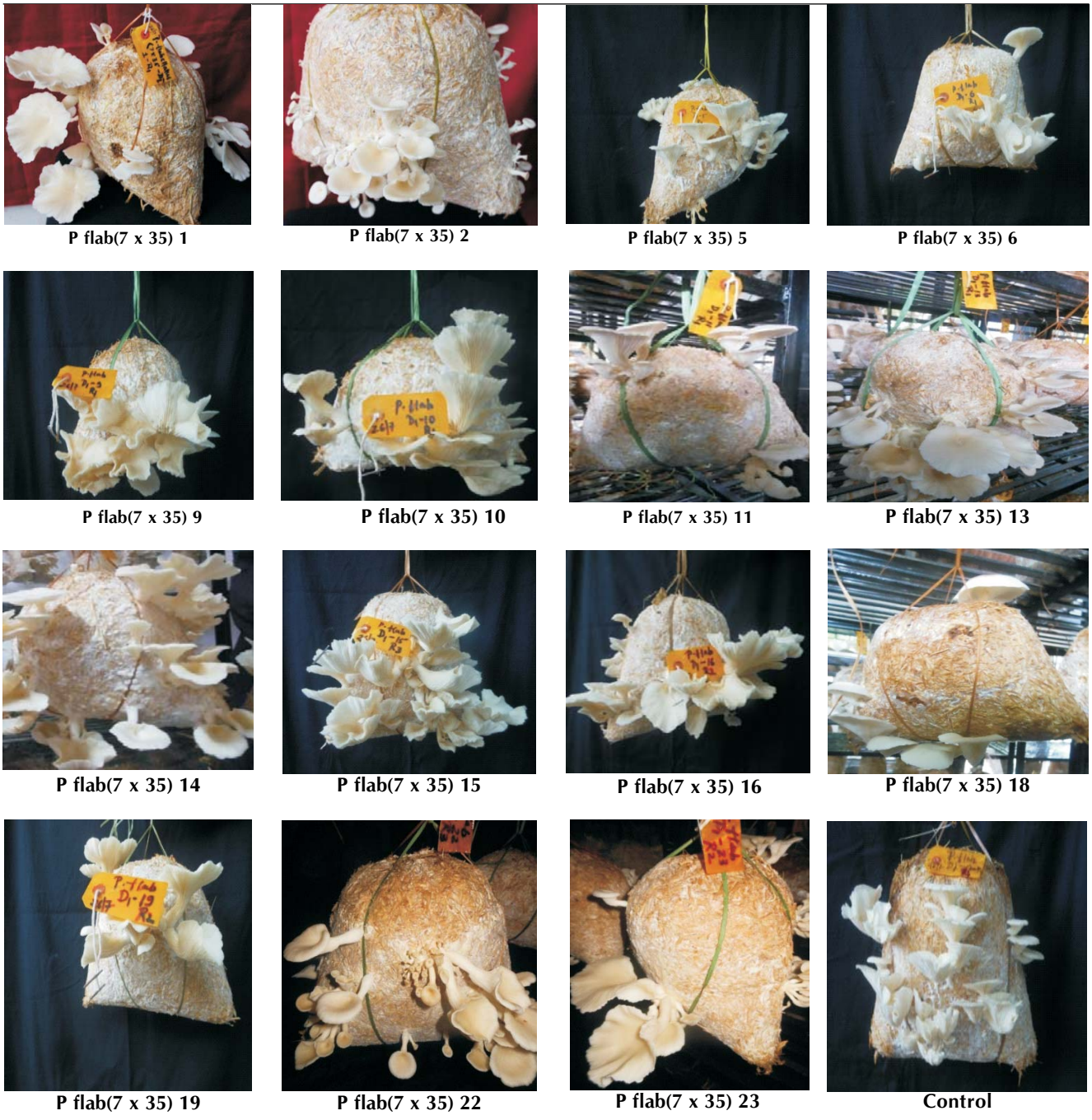


Figure 2: Evaluation of dikaryons of P flab (7 x 35) for fruiting body formation from the confluence region of two compatible mating partners of *P. flabellatus*

Dextrose Agar medium (till full growth of any one of the isolates) and were evaluated for cultural characteristics (colour of the fungus, type and patterns of growth (White, milky white, dull white, compact, thick, thin with regular or irregular margin). The isolates varied for their colour and type and patterns of growth. Different shades of white with ranged from absolute white, dull white, milky white, creamish, grayish, white with yellow pigmentation were observed in the vegetative growth of the single basidiospore isolates (derived from *Pleurotus flabellatus*).Similarly different patterns of mycelial growth were observed in fully attained growth of all monokaryons which varied from flat, thick, thin, and compact with wavy, regular or irregular margin, thick or thin at center and the formation of concentric rings. The major aim of breeding is to combine the desirable characteristics from different strains and create variability in the existing germplasm. Variation in colony characters of *P. flabellatus* was recorded in PDA produced bright white colour colonies. The mycelial growth in PDA medium appeared in the form of rings. Morphological characters like prominent interaction in the contact zone,

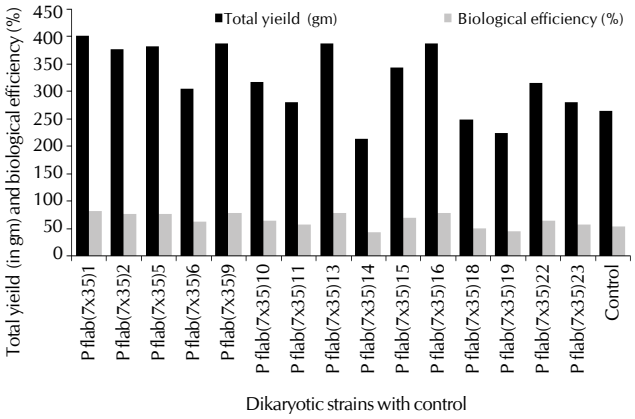


Figure 3: Total yield (in gm) and biological efficiency (%) of 15 dikaryons derived from a cross of P flab (7 x 35) of *P. flabellatus* with control

increased rate of mycelium growth, better colony morphology have been used as the morphological markers in previous studies for breeding purposes (Gharehaghaji *et al.,* 2007; Kavousi *et al.,* 2008). Radial growth of dikaryons has been reported to depend on the growth of the monokaryons that are composing the dikaryons (Wang and Anderson 1972). Similarly, in the present study, variability in the morphological features offer better prospects for the strain improvement through hybridization based on the crossing of non-fertile homokaryotic lines.

Identification of mating types of single basidiospores

Single basidiospores of *Pleurotus* are sterile in nature and can form dikaryotic mushroom only after mating with the other compatible isolate (Brown and Casselton 2001). Mating type in heterokaryotic mushrooms like *Pleurotus* is controlled by two genetic loci named as A and B (Kothe 2001). In nature, this is only the case if two cells of different mating types have fused to combine the different proteins in one cytoplasm (Kothe 2001). Using these criteria we identified mating types of all the isolates by two-point culture technique (Fig. 1). Total of 1229 possible pairs were made between the isolates of *P. flabellatus*. It may be the consequence of either the similar mating type of all the isolates or inability of the isolates to fuse with each other under laboratory conditions (Ikeda *et al.,* 2002). The compatible pair which formed a very thick tuft at the confluence region was selected as tester strains to identify the mating types of all the other isolates. P flab 7 and P flab 35 were identified as tester strains for *P. flabellatus* isolates. P flab7 and P flab35 designated as AxBx and AyBy, respectively. Out of 49 monokaryotic isolates tested against both P flab7 and P flab35, only 1 were compatible with the P flab7 but not with the P flab 35. Mating type of AyBy, opposite to that of P flab 7 (AxBx) was assigned to 1 isolates. Whereas, AxBx was given to the isolates that were incompatible with P flab7 interestingly, exact mating type cannot be assigned to these isolates. They can have either AxBy or AyBx mating type. Similarly, 3 isolates showed compatibility with P flab 35(Table 1). Besides mating type genes, many other can be responsible for self versus non self recognition thus leading to incompatibility between the two isolates (Kronstad and Staben 1997). Mating type genes and other compatibility factors are now used in mating-type-assisted breeding programs for producing economical and edible mushroom species (Kothe 2001).

Dikaryon isolation and evaluation of the spawn for yield and biological efficiency

Iner-strain/intra-species hybridization involves isolation of single spores and intermating of the single spore isolates (May and Royse 1982). Two compatible mating pairs were selected (P flab 7 x P flab 35 and P flab35 x P flab 120) for dikaryon islation from prominent tuft in the confluence region of the omponent isolates. A total of 30 dikaryons (15 from P flab 35 x flab 120, and 15 from the P flab 7 x P flab 35) from the cntact zone were isolated. All the two crosses produced fruiting which is possible only when mycelia of two single basidiospores of opposite mating type fuse and form the dikaryon (Brown and Casselton, 2001). Thirty dikaryons fifteen from P flab35 x P flab120 cross and fifteen from and P flab 7 x P flab 35 in three replicated bags were observed for their spawn run period, pinhead initiation, size of sporophore, no. of sporophore and yielding ability (Table 2 and 3).

Observation pertaining to sixteen bags has been presented in Table 2. Dates of different stages of growth of the dikaryotic cultures on wheat straw indicated that to attain full growth/ spawn run (DAI) was required 11.33 to 14.67 days for all strains of P flab (7 x 35) and 12 to 15.33 days for all strains of P flab (35 x 120). Pin head initiation (DAS) was appeared within 4 to 6 days in all strains of P flab (7 x 35) and 4.33 to 6.67 days in all strains of P flab (35 x 120). Time period taken by the dikaryons for mycelial growth (spawn run period) is very less as reported earlier for *P. sajor-caju* (Shahid *et al.*, 2006). Observations regarding the date of full spawn run, date of pinhead formation and yield in subsequent three pickings were recorded within a time of 1 month from the date of full spawn run (Table 3 and 4). Though the dikaryons were isolated from the same set of two compatible mating pairs, but the dikaryons showed variation in number of days taken for full spawn run, pin-head formation, size of sporocarp, no. of sporophores and total yield. Similar findings were obtained by Sahu *et al*. (2014) on *P. eous*, which were taken 9.33 days for spawn run and 13.33 days for primordial initiation on wheat straw. Pileus diameter was found highest (7.85 cm) in strain of P flab (7 x 35) 1, stipe length was highest (3.45 cm) in strain of P flab (7 x 35) 15, stipe diameter was found maximum in strain of P flab (7 x 35) 16 (1.10 cm) of *P. flabellatus*. Similarly highest pileus diameter was found in strain of P flab (35 x 120) 13 (8.6 cm), highest stipe length was found in strain of P flab (35 x 120) 13 (3.66 cm) and highest stipe diameter was reported in strain of P flab (35 x 120) 13 (1.05 cm). Sawale (2004) reported average length and width of sporophores to be 5.23 cm and 3.88 cm respectively in different *Pleurotus* spp. Maximum numbers of sporophores was produced by Strain of P flab (7 x 35) 9 (24.44) and strain of P flab (35 x 120) 5 (26.11). Highest Total yield and Biological Efficiency was found in strains of P flab (7 x 35) 1 (401.67 gm, 80.33%). and strain P flab (35 x 120) 2 (447 gm, 89.41%) of *P. flabellatus* on wheat straw. According to Namdev and Thakur (2002), wheat straw (427 gm with 68% BE) is the best substrate for cultivation of *P. flabellatus*. Sawale (2004) the fresh yield of wild *Pleurotus* spp. was significantly superior recorded on wheat straw (430 g) and paddy straw (371 g).


P flab(35x 120) 1


P flab(35x 120) 2


P flab(35x 120) 5


P flab(35x 120) 6


P flab(35x 120) 9


P flab(35x 120) 11


P flab(35x 120) 12

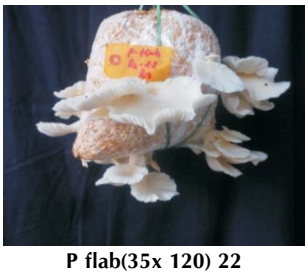

P flab(35x 120) 13

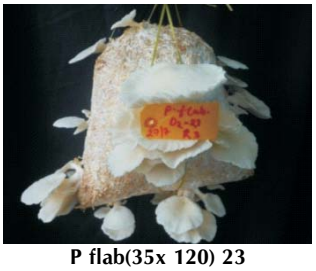

P flab(35x 120) 15


P flab(35x 120) 17


P flab(35x 120) 19


P flab(35x 120) 20


P flab(35x 120) 22


P flab(35x 120) 23


P flab(35x 120) 24

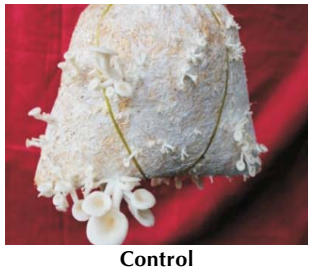

Control

Figure 4: Evaluation of dikaryons of P flab (35 x 120) for fruiting body formation from the confluence region of two compatible mating partners of *P. flabellatus*

Gupta *et al*. (2011) cultivated different strains of *P. sajor-caju* on paddy straw. They found strain (Ps13xPs47)1b to produce maximum harvest on paddy straw substrate (385 g/kg dry straw, 76% B.E.). Sahu *et al*. (2014) reported that *P. eous* produced higher yield 525.55 gm/kg dry wheat straw substrate with B.E. 78.79 %. Number of dikaryons tested in this study is very less but all the dikaryons are showing better traits as that can be the basis for strain improvement. Such type of strategies with special reference to hyphal anastomosis has been reported for improving breeding potentials of edible mushrooms (Jandaik 1997; Kothe 1996; Pahil 1997) in both the *Agaricus* and *Pleurotus* species. Various *Pleurotus* spp. reflect much of the genetic variation within which the tools of selection, breeding and genetic engineering can be applied to find strains adapted to wider range of the substrate, environment and cultivation methods (Kapoor *et al*., 1996; Ikegaya 1997). In this study, development and evaluation of the interstrainal hybrids was performed at a very small scale, and it seems worthwhile to apply this method at broader level to the other strains of *Pleurotus* spp. that have special traits. Also the knowledge of the morphological and genetic differences along with the mating type of the monospores can provide the basis for selection of the isolates for developing high yielding hybrids.

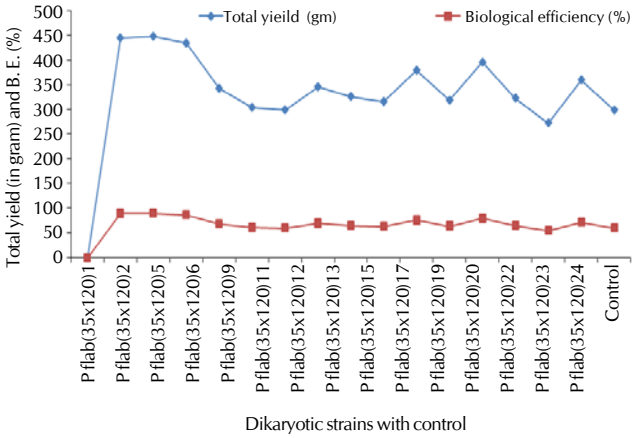


Figure 5: Total yield (in gm) and biological efficiency (%) of 15 dikaryons derived from a cross of P flab (35x120) of *P. flabellatus* with control

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REFERENCES

Bisko, N. A. and Kholodny, N. G. 1989. Selection of *Pleurotus ostreatus* (*Jacq. Fr.)* Kumm. In: Abstracts of international symposium on mushroom biotechnology. *Nanjing, China.* p. 21

Brown, A. J. and Casselton, L. A. 2001. Mating in mushrooms: increasing the chances but prolonging the affair. *Trends. Genet.* **17:** 393-400.

Fritsche, G. 1981. Some remarks on the breeding, maintenance of strains and spawn of *Agaricus bisporus* and *Agaricus bitorquis*. In: Mushroom Science XI. Proceedings of the eleventh international scientific congress on the cultivation of edible fungi,Australia, pp. 367-385.

Gharehaghaji, A. N., Goltapeh, E. M., Masiha, S., Gordan, H. R. 2007. Hybrid production of oyster mushroom *Pleurotus ostreatus* (Jack; Fries) Kummer. *Pakistan J. Biol. Sci* .**10:** 2334-2340.

Gupta, B. *et al.* 2011. Molecular characterization and mating type analysis of oyster mushroom (*Pleurotus spp.)* using single basidiospores for strain improvement. *World. J. Microbiol Biotechnol.* 2011. **27(1):** 1-9.

Nakamura, H. and Matsumoto, N. 2002. Mycelial incompatibility operative in pairings between single basidiospore isolates of Helicobasidium mompa. *Mycol. Res.* **107:** 847-853.

Ikegaya, N. 1997. Breeding and cultivation of shiitake (*Lentinus edodes*) mushrooms. *Food Res. Int.* **13:** 335-356.

Jaindaik, C. L. 1997. History and development of Ikeda, K. *Pleurotus*: cultivation in the world and future prospects. In: Upadhyay RC, Singh SK, Rai RD (eds) Current vistas in mushroom biology and production. *Mushroom Society of India,* Solan, pp.181-191.

Kapoor, M., Sodhi, H. S. and Dhanda, S. 1996. Strategies for strain improvement in *Pleurotus spp. Mushroom Res.* **5:** 57-66.

Kavousi, H. R., Farsi, M. and Shahriari, F. 2008. Comparison of random amplified polymorphic DNA markers and morphological characters in identification of homokaryon isolates of white button mushroom (*Agaricus bisporus*). *Pakistan J. Biol. Sci.* **11:** 1771-1778.

Kligman, A. M. 1943. Some cultural and genetic problems in the cultivation of the mushroom *Agaricus compestris. Fr. Am. J. Bot.* **30:** 745-763.

Kotasthane, A. S. 2003. A simple technique for isolation of Xanthomonas oryzae pv oryzae. *J. Mycol. Plant Pathol.* **33:** 277-278.

Kotasthane, A. S. and Singh, U. S. 2000. Simple technique for single spore isolation and a modified two-point inoculation technique of mating in test tubes. *J. Mycol. Plant Pathol.* **30:** 366-369.

Kotasthane, A. S. and Agrawal, T. 2010. A simple technique for single spore isolation of micro sized fungal spore. *J. Mycol. Pl. Pathol.* **40(3):** 311-313.

Kothe, E. 1996. Tetrapolar fungal mating types: sexes by the thousands. *FEMS Microbiol Rev.***18:** 65-87.

Kothe, E. 2001. Mating-type genes for basidiomycetes strain improvement in mushroom farming. *Appl. Microbiol Biotechnol.* **56:** 602-612.

Kronstad, J. W. and Staben, C. 1997. Mating type in filamentous fungi. *Annu. Rev. Genet.* **31:** 245-276.

Kues, U. and Liu, Y. 2000. Fruiting body production in basidiomycetes. *Appl. Microbiol Biotechnol.* **54:** 141-152.

Kumar, S. 1987. Studies on some mushroom families in the North Western Himalayas. Dissertation, H.P. University, Shimla, India

Kumar, S. and Munjal, R. L. 1981. Studies on evolving new strains of the cultivated mushroom (*Agaricus brunnescens* Peck) by single spore isolation method. *Indian J. Mush.* **7:** 37-42.

Kyung-Ho Ma., Lee Gi-An and Lee Sok-Young 2009. Development and characterization of new microsatellite markers for the oyster mushroom (*Pleurotus ostreatus*). *J. Microbiol Biotechnol.* doi. **10:** 4014/jmb.0811.604.

May, B. and Royse, D. J. 1982. Confirmation of crosses between lines of *Agaricus brunnescens* by isozyme analysis. *Exp. Mycol.* **7:** 283-292.

Namdev, J. K. and Thakur, M. P. 2002. Studies on Radial Growth and yield of *Pleurotus flabellatus* as influenced by substrates and month of cultivation. *J. Mycol. Pl. Pathol.* **32(2):** 281.

Nasim, G., Malik, S. H., Bajwa, R., Afzal, M. and Mian, C. G. 2001. Effect of three different culture media on mycelia growth of oyster Chinese mushroom. *J. Biol. Sci.* **1:** 1130-1133.

Pahil, V. S. 1997. Strain improvement in tropical *Agaricus*: Techniques selection of genetically diverse parents for hybridization in *Agaricus bisporus*. *Mushroom Res.* **12:**19-26.

Perisamy, K. and Nataranjan, K. 2003. Strain improvement of *Pleurotus djamor (Fr.) boedijn var. roseus* corner using conventional breeding technique. In: Upadhyay RC, Singh SK, Rai RD (eds) Current vistas in mushroom biology and production. *Mushroom Society of India, Solan*, pp. 35-46.

Sahu, S. K. 2014. Screening of suitable grains substrates for Spawn development, growth and yield of *Pleurotus eous. American International J. Research in Formal, Applied and Natural Sciences.* **5(1):** 86-89.

Sawale, V. V. 2004. Comparative Studies on wild pink oyster mushroom, *Pleurotus* sp. and *Pleurotus eous*. M.Sc. Thesis submitted to IGAU, Raipur (C. G.)

Shahid, M. N., Abbasi, N. A. and Saleem, N. 2006. Effect of different methods of compostpreparation and lime concentration on the yield of Pleurotus sajor-caju. *Int. J. Agri. Biol.* **8:** 129-131.

Sharma, T. C., Sharma, I. and Patiri, B. N. 2010. Wild edible mushrooms used by some ethnic tribes of Western Assam. *The Bioscan.* **3:** 613-625.

Sharma, S., Khanna, P. K. and Kapoor, S. 2013. Effect of supplementation of Wheat bran on the production of shiitake (*Lentinus edodes* (berk) peglar) using wheat straw and saw dust substrates. *The Bioscan.* **8(3):** 817-820.

Sigoillot, C., Camarero, S., Vidal, T., Record, E., Asther, M., Perez-Boada, M. 2005. Comparison of different fungal enzymes for bleaching high-quality paper pulps. *J. Biotechnol.* **115:** 333-343.

Sonnenberg, A. S. M. 2000. Genetics and breeding of Agaricus bisporus. *Mush. Sci.* **15:** 25-39.

Wang, S. S. and Anderson, N. A. 1972. A Genetic analysis of sporocarp production in *Pleurotus ostreatus*. *Mycologia* **. 64:** 521-528.

Yadav, M., Tripathi, Y. K., Verma, R. N., Upadhyay, R. C. and Dhar, B. L. 2003. Molecular breeding for development of genetically improved strain and hybrids of *Agaricus bisporus*. In: Upadhyay RC, Singh SK, Rai RD (Eds) Current vistas in mushroom biology and production. *Mushroom Society of India, Solan*, pp. 261-274.