

## Development of Intraspecific Hybridization of *Pleurotus flabellatus* for Better Yield and Nutrition

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### ABSTRACT

#### Keywords

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The research was framed to develop intraspecific hybrids of *Pleurotus flabellatus* having earliness in production, better nutritional qualities and higher yield compared to their parent strains. Fifteen monosporus cultures of *P. flabellatus* were isolated by spore print or serial dilution and or hyphal tip fragmentation techniques. Thirty four intraspecific hybrids were developed from different cross combinations of parents of *P. flabellatus*. Out of 34 intraspecific hybrids, 20 hybrids showing higher growth rate in malt extract media were selected to study the qualitative and quantitative responses during cultivation. Spawn running was faster in PF×PF33, PF×PF25, PF×PF26 and PF×PF27. Pinhead formation was quickest in PF×PF33, PF×PF24 and PF×PF7. Comparing all parameters of growth PF×PF1, PF×PF5, PF×PF13 and PF×PF33 were found most efficient. Nitrogen and protein concentration was good enough in PF×PF4 and PF×PF22. Considering all the quantitative parameters like biological efficiency, yield of 1st and 2nd harvest, PF×PF24, PF×PF33, PF×PF10, PF×PF9 and PF×PF22 may be selected for higher production.

### Introduction

*Pleurotus* which are white rot fungi of phylum basidiomycota having high saprophyte colonizing ability, can grow on any agricultural waste and show great diversity to the varying agro-climatic condition. This ability of utilizing agricultural waste into valuable product gives more importance than any other cultivated mushroom. In India, Bano and Srivastava (1962) first standardized the cultivation of *Pleurotus flabellatus* and first domesticated species of *Pleurotus* as *P. ostreatus*. Later, *P. sajor-caju* come in highlight for cultivation in

1974 when Jandaik and Kapoor first reported its cultivation on banana pseudo stem chopped with paddy straw.

Dikaryotization of selective strains is a very important tool in strain improvement for bringing genetic recombination and developing somatic hybrids which has been used by several workers to develop new strains of *Pleurotus* with the findings of fast colonizing ability which lead to early flushing of the fruit body with good shape, size, low mortality rate of the bud, good color of the

pelius and the high protein content (Bahukhandi and Shrma, 2002; Jaswal *et al.*, 2013). In this studies intraspecific hybridization of *P. flabellatus* were done through selective dikaryotization with an aim to assemble the best combination of genes into one individual hybrid with higher productivity along with better nutritional quality differing to their parents.

## **Materials and Methods**

### **Isolation of monosporus culture**

Following 2 different methods were followed for producing monosporus culture. Spore print method: Petersen and Ridly (1996) method was followed for the single spore isolation of both PF and PSCC (Plate 1).

Serial dilution method: Bahukandi and Sharma (2002) described the method and the same was followed with little modification for the isolation of single spore.

### **Intraspecific hybridization: (Dual plate techniques)**

Mycelial disc of three millimeter diameter from periphery of seven days old of different monosporus cultures were placed in the two opposite sides of the Petriplates containing malt extract agar media and were incubated for 3-4 days at 25±10C(Plate 3).

Small inoculums was taken by chopping from the meeting points of two different isolates (Plate 4), dikaryotization was further confirmed by the formation of hyphal bridge or by the presence clamp connection (Plate 5)

### **Parentage and intraspecific hybrids of *P. flabellatus***

Out of 34 intraspecific hybrids, 20 hybrids showing higher growth rate in malt extract

media were selected to study the qualitative and quantitative responses during cultivation (Table 1).

### **Cultivation of *P. flabellatus* hybrids**

Jodon and Royse (1979) given method were followed for making of spawn of hybrids culture.

### **Preparation of mushroom cylinder**

3kg sterilized paddy straw was filled from the opening side of polypropylene bag for about 3inch-4inch and the layer of spawn of individual strain was spread by hand and again same process of alternate layer filling of straw and spawn was practiced for 4-5 times. For each complete cylinder 200gm of spawn was added. Small hole was made over the polypropylene bag for aeration and cotton was plugged in the holes to protect from contamination by pathogens invasion by the insects.

### **Quantitative estimation of Hybrid mushroom:**

Five hundred gram freshly harvested fruit body mostly from the first flush were taken as a sample for calculating the amount of dry matter. Freshly harvested mushroom was kept in a hot air oven for drying at 60<sup>0</sup>C for 3 days and the individual dried sample were weighed for the calculation of dry weight of mushroom. Ashraf *et al.*, (2013) formula was followed for the calculation of moisture percent of individual sample of mushroom.

$$\frac{\text{initial weight of sample} - \text{dried weight of sample}}{\text{weight of sample}} \times 100$$

Most of the worker calculated biological efficiency as fresh weight of mushroom in relation to the dry weight of substrate but

Oliveira *et al.*, (2014) suggested that the biological efficiency will be more accurate if we compare dry weight of yield to the dry weight of substrate, so biological efficiency percent was calculated by using the formula:

$$\frac{\text{dry weight of mushroom}}{\text{dry weight of substrate}} \times 100$$

### Qualitative estimation of hybrid mushroom

Total protein contain in dry matter of mushroom was estimated following Lowry's method (1951), Nitrogen in culture broth was determined by KEL PLUS nitrogen estimation system (Jackson, 1973).

### Results and Discussion

#### Spawn running, pinhead formation 1<sup>st</sup> and 2<sup>nd</sup> flush

The earliness to reach different stages such as completion of spawn run in substrate, pinhead formation, production of 1<sup>st</sup> and 2<sup>nd</sup> harvestable fruit bodies within the intraspecific hybrids were calculated. The results revealed that successful spawn run in the substrate took minimum duration in PF×PF33, PF×PF25, PF×PF26, PF×PF5 and PF×PF27 (16-17 days from spawn inoculation). No spawn run was observed in PF×PF19 and PF×PF20 when they were inoculated in the rice straw. It indicated the

complete abortiveness of these strains and hence excluded from selection.

Likewise, PF×PF27 although, showed earliness of spawn run, but no pinhead formation was recorded which indicated the abortiveness of the strains at this stage. Pinhead formation was found earlier in PF×PF33, PF×PF24, PF×PF7 (2-3 days after completion of spawn run, Table 2, Plate 7). First flush of fruits bodies was earliest in PF×PF2, PF×PF4, PF×PF7, PF×PF9, PF×PF11, PF×PF12, PF×PF17 (2 days from the pinhead formation, Table 2, Plate 8) which was equal to its parent. Variation in earliness of 2<sup>nd</sup> flush within the hybrids was found and was ranged between 7-14 days after 1<sup>st</sup> flush.

It is evident from the results of cultivation of intraspecific hybrids of *Pleurotus* sp. that spawn running took 2-3 weeks after inoculation in suitable substrate which was in agreement with Tan (1981). Ahmad (1986) stated that *P. ostreatus* completed spawn running in 17-20 days on different substrates and time for pinheads formation was noted as 23-27 days. Quimio (1978) who reported that fruiting bodies 3-4 weeks after inoculation of spawn. Saha *et al.*, (2004) reported that the earliest spawn running of 16-17 days was found in wheat straw and wheat straw supplemented with the leaves.

Plate 1



Plate 2



Plate 3



Plate 4



Plate 5

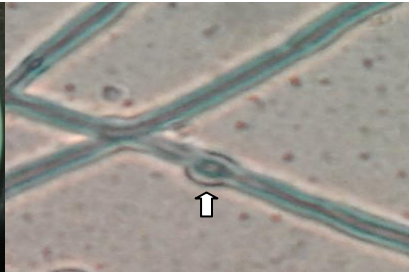


Plate 6



Fig 1 & Fig 2: isolation of single spore through spore print, Fig 3: Somatic hybridization in *Pleurotus*, Fig 4: Isolation of dikaryotic mycellium from meeting point, Fig 5: Microscopy for presence of clamp connection to ascertain hybridization, Fig 6: Production of spawn in conical flask.

Plate 7



Plate 8



Plate 9



Plate 10



Plate 7 Spawn running of Intraspecific Hybrids, Plate 8: Pin Head formation of Intraspecific Hybrids, Plate 9 & Plate 10: Fruit body production of Intraspecific Hybrids

**Table.1** PFm: *P. flabellatus* monosporus culture. PFm × PFm: different monosporus culture crossed with each another

Parentage	Hybrids
PF9m × PF10m	PF×PF1, PF×PF2, PF×PF3,
PF9m × PF11m	PF×PF4, PF×PF5
PF9m × PF1m	PF×PF6, PF×PF7, PF×PF8,
PF10m × PF9m	PF×PF9, PF×PF10, PF×PF11,
PF10m × PF11m	PF×PF12, PF×PF13, PF×PF14,
PF10m × PF1m	PF×PF15, PF×PF16, PF×PF17,
PF11m × PF9m	PF×PF18, PF×PF19, PF×PF20,
PF11m × PF10m	PF×PF21, PF×PF22, PF×PF23,
PF11m × PF10m	PF×PF24, PF×PF25, PF×PF26
PF1m × PF9m	PF×PF27, PF×PF28, PF×PF29,
PF1m × PF10m	PF×PF30, PF×PF31,
PF1m × PF11	PF×PF32, PF×PF33, PF×PF34,

**Table.2** ICRN: intraspecific crosses number, SR: Duration (days) between Spawning to spawn running, PH: Duration (days) between Spawn running to pinhead formation of fruit body, 1<sup>ST</sup>F: Duration (days) between pinhead formation to first flushes of fruit body, 2<sup>ND</sup> F: first flushes to second flushes of fruit body, Fwt: fresh weight (gram) of total mushroom yield till 2<sup>nd</sup> flushes, Dwt: dry weight (gram) of total mushroom yield till 2<sup>nd</sup> flush, BE%: biological efficiency on the basis of dry weight of mushroom. Most%: moisture content in mushroom

ICRN	SR	PH	1 <sup>ST</sup> F	2 <sup>ND</sup> F	Fwt	Dwt	BE%	Most%
PF X PF1	18	4	3	8	962	98.57	9.86	89.75
PF X PF2	19	4	2	10	921	91.25	9.13	90.09
PF X PF4	21	6	2	8	891	86.94	8.69	90.24
PF X PF5	17	4	3	7	940	99.68	9.97	89.40
PF X PF7	23	3	2	7	931	91.41	9.14	90.18
PF X PF9	21	5	2	8	985	102.46	10.25	89.60
PF X PF10	21	6	3	8	1074	108.11	10.81	89.93
PF X PF11	24	5	2	7	856	87.18	8.72	89.82
PF X PF12	21	6	2	10	848	80.27	8.03	90.53
PF X PF13	21	4	4	9	937	90.78	9.08	90.31
PF X PF16	23	5	3	8	900	88.22	8.82	90.20
PF X PF17	21	7	2	10	803	80.83	8.08	89.93
PF X PF19	0	0	0	0	0	0.00	no	0.00
PF X PF20	0	0	0	0	0	0.00	no	0.00
PF X PF22	22	7	3	8	992	93.84	9.38	90.54
PF X PF24	19	3	4	14	1150	117.44	11.74	89.79
PF X PF25	17	7	4	9	666	66.80	6.68	89.97
PF X PF26	17	4	3	13	871	85.46	8.55	90.19
PF X PF27	17	0	0	0	0	0.00	no	0.00
PF X PF33	16	2	3	10	1079	109.58	10.96	89.84
PF control	24	6	2	8	777	75.28	7.53	90.31

**Table.3** Nitrogen % and protein % present in dry matter of *P. flabellatus* hybrids

<b>CROSSES</b>	<b>NITROGEN (%)</b>	<b>PROTEIN (%)</b>
PF X PF1	3.64	35.50
PF X PF2	3.08	36.25
PF X PF3	0	0.00
PF X PF4	3.36	35.75
PF X PF5	3.78	33.75
PF X PF7	2.8	30.50
PF X PF9	2.94	29.50
PF X PF10	3.08	37.00
PF X PF11	3.22	37.25
PF X PF12	2.8	26.75
PF X PF13	3.64	35.25
PF X PF16	3.08	30.25
PF X PF17	2.94	32.25
PF X PF19	0	0.00
PF X PF20	0	0.00
PF X PF22	3.64	37.75
PF X PF24	3.08	34.25
PF X PF25	2.94	28.13
PF X PF26	3.08	35.75
PF X PF27	0	0.00
PF X PF33	2.94	30.25
PF control	2.94	25.50

**Fresh weight, dry weight and moisture % of intraspecific hybrids**

Highest fresh weight and dry weight was found in PF X PF33, PF X PF24, PF X PF10 of about 1150 to 1074 and 117.44 to 108.11 gram per cylinder till 2<sup>nd</sup> flushes which will match with the result of Saha *et al.*, (2004) who recorded that the average yield of *P. ostreatus* was 210.6 – 646.9 gram per flushes in different substrate. Most of the hybrids were found with good yielding capacity as compare to parent. Similar kind of result was found by Kaur *et al.*, (2007) among monokaryons of *Pleurotus florida* PAU-5, Out of which PFJ 11 yielded out the parent while average weight of fruit bodies was higher in PFJ 13 (9.9 g) as compared to parent (9.6 g). Spawn run was recorded faster in PFJ 11 (39 days) and PFJ14 (41 days) with respect

to that of the parent (48 days). Peng *et al.*, (2001) also obtained some highly productive strains through mating which took 54.4 to 60 days from inoculation to the end of the crop and they reacted differently to the composition of substrate in the aspect of productivity. BE% is directly related with the yield of the mushroom, all the above hybrids having high yielding capacity contain greater value of BE% of about 11.7 to 10. BE% was found in higher value as comparing with the literature which are varying from 4.08 to 5.03% (Bhatti *et al.*, 2007; Holtz *et al.*, 2009; Furlan *et al.*, 2008, Oliveira *et al.*,2007;). Moisture % did not shows any variation as compare to parents which was around 89% - 90%, which can correlate will almost all researcher who found moisture content of about 85% - 95% (Tewari, 1986; Ahmed *et al.*, 2013).

### Nitrogen % and protein % present in *P. flabellatus* hybrids

Almost all the hybrid had showed the positive relations between the Nitrogen and protein content. PF×PF5 showed higher nitrogen (3.78%) which was closely followed by PF×PF1, PF×PF13, PF×PF22 (3.64%). All the hybrids except PF×PF7 and PF×PF12 shows the higher nitrogen content as compare to the parent *P. flabellatus* (2.94%). PF×PF9, PF×PF17, PF×PF25, and PF×PF33 showed same amount of nitrogen with the parent. Protein content was found highest in PF×PF22, PF×PF11, PF×PF10, PF×PF2 (37.75-36 mg/ g of fresh mushroom). All the hybrids showed the higher content of protein as compare to the parent (25mg/ g of fresh mushroom). When two parameters were taken into consideration, PF×PF4 and PF×PF22 have the highest nutritive value which was followed by PF×PF1, PF×PF13, PF×PF5, PF×PF2, PF×PF26, PF×PF10, PF×PF11, PF×PF24. *Pleurotus* species are considered to be one of the most efficient producers of food protein (Ogundana and Okogbo, 1981). Manzi *et al.*, (2001) reported that chemical composition of mushroom will varies from species to species, it also depends on different substrate, atmospheric condition, age and part of fructification. Bernas *et al.*, (2006) showed that the total nitrogen content ranged between 3.3-4.0g and proteinaceous nitrogen was 2.0-2.2g per 100 g dry weight of mushroom. Ahmed *et al.*, (2013) evaluated yield and chemical composition of oyster mushroom strains newly introduced in Bangladesh. The strains were *P. high-king* (strain PHK), *P. ostreatus* (strain PO2), and *P. geesteranus* (strains PG1 and PG3). They observed that different strains of same *Pleurotus* sp differed in N, P, K, Na and protein content (Table 3). The results are in accordance with Bhattacharya *et al.*, (2015) who suggested that the nitrogen content of *P. ostreatus* are 4.03% - 4.52%. Considering all the

quantitative parameters like biological efficiency, yield of 1st and 2nd harvest, PF×PF24, PF×PF33, PF×PF10, PF×PF9 and PF×PF22 may be selected for higher production.

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