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Development of Interspecific Hybrids of *Pleurotus sajor-caju* and *P. flabellatus* for Better Yield

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ABSTRACT

Existence of variability in morphological traits and growth rate of mycelium of homokaryotic single basidiospores can be exploited for the development of interspecific hybrids of mushroom. To explore this, interspecific hybrids of *P. flabellatus, P. sajor-caju* having earliness in production, better nutritional qualities and higher yield as compared to their parent strains tried at Uttar Banga Krishi Viswavidyalaya. Eighteen and six monosporus culture of *P. flabellatus, P. sajor-caju*, respectively were isolated by spore print or serial dilution or hyphal tip fragmentation techniques. Twenty interspecific hybrids were developed from different cross combinations of parents *P. flabellatus* and *P. sajor-caju*. The hybrids were grown on malt extract agar media. Highest growth rate shown by the hybrids in media were selected to study qualitative and quantitative response during cultivation. In cultivation spawn running and pin head formation was faster in PF × PSC5 and PF × PSC7. Comparing all the parameters, growth of PF × PSC6 and PF × PSC8 were found to be more efficient. Nitrogen and protein content was good enough in PF × PSC4, PF × PSC7, PF × PSC5 and PF × PSC10. Regarding all the quantitative parameters of the hybrids, biological efficiency and yield of 1st and 2nd harvest of PF × PSC6 and PF × PSC8, PF × PSC5, PF × PSC10 and PF × PSC3 were better than the rest hybrid combinations. In interspecific hybrids of *P. flabellatus* and *P. sajor-caju*, the highest yield and biological efficiency was found in PF × PSC6 combination and may be selected for better mushroom production.

Keywords *Pleurotus flabellatus, Pleurotus sajor-caju*, Monosporus culture, Interspecific hybridization, Dikaryotization.

INTRODUCTION

Mushroom in general and *Pleurotus* 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 chloresterol whereas, rich in proteins, minerals, vitamins and fibers (Nasim *et al.* 2001). However, *Pleurotus* is ranked second next 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 favourite 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,

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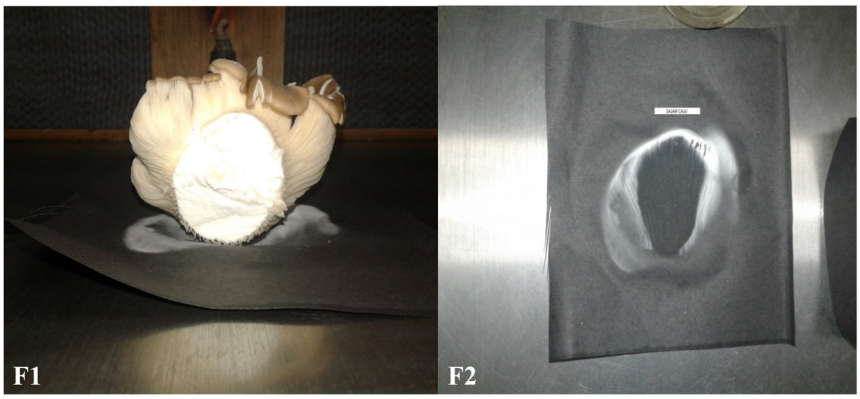

Fig. 1. Discharge of spore from *P. sajor-caju.* **Fig. 2.** Spore print from fruit body of *P. sajor-caju.*

better quality, texture, color and taste to meet the rising demands of the increasing population. Dikaryotization of selective strains is very important tool in strain improvement for bringing genetic recombination and developing somatic hybrids which have been used by several workers to develop new strains of *Pleurotus* with the findings of fast colonizing ability which leads to early flushing of the fruit body with good shape, color of the pileus and high protein content (Bahukhandi and Sharma 2002, Jaswal *et al.* 2013). In this study, interspecific hybridization of *P. flabellatus* and *P. sajor-caju* was done through selective dikaryotization with an aim to assemble the best combination of genus into one individual hybrid with higher productivity along with better nutritional quality differing to parents.

MATERIALS AND METHODS

Place of experimentation : The laboratory experiments were done in Research Laboratory, Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar.

Table 1. Parentage and interspecific hybrids of *P. flabellatus* and *Pleurotus sajor-caju*.

Parentage	Hybrids
PF8M × PSC3M	PF × PSC1, PF × PSC2, PF × PSC3, PF × PSC4
PF8M× PSC5M	PF × PSC5, PF × PSC6
PF11M× PSC3M	PF × PSC7, PF × PSC8, PF × PSC9
PF11M× PSC5M	PF × PSC10, PF × PSC11
PF16M× PSC3M	PF × PSC12, PF × PSC13, PF×PSC14
PF16M× PSC5M	PF × PSC15, PF × PSC16
PF17M × PSC3M	PF × PSC17, PF × PSC18
PF17M× PSC5M	PF × PSC19, PF × PSC20

Isolation of Monosporous culture of *P. flabellatus, P. sajor-caju* : Isolation of monosporous culture is one of the most important steps to develop homokaryons before aiming hybridization for strain improvement. The method proposed by Petersen and Ridley (1996) was followed for the single spore isolation for both *P. flabellatus* and *P. sajor-caju.* (Figs. 1–2).

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

Interspecific hybridization (Dual plate techniques) : Interspecific hybridization was done in petri plates using malt extract agar media. Mycelial disc of three-millimeter diameter from periphery of 7 days old of homokaryotic cultures were placed in two opposite sides of the petri plates containing media and incubated for 3-4 days at 25°C. Small amount of inoculum was taken by chopping from the meeting points of two different isolates. Dikaryotization was further confirmed by the presence of clamp connections or by hyphal bridges (Figs. 3–4).

Parentage and interspecific hybrids : 20 hybrid strains of *P. flabellatus* and *P. sajor-caju* showing higher growth rate in malt extract media were select-

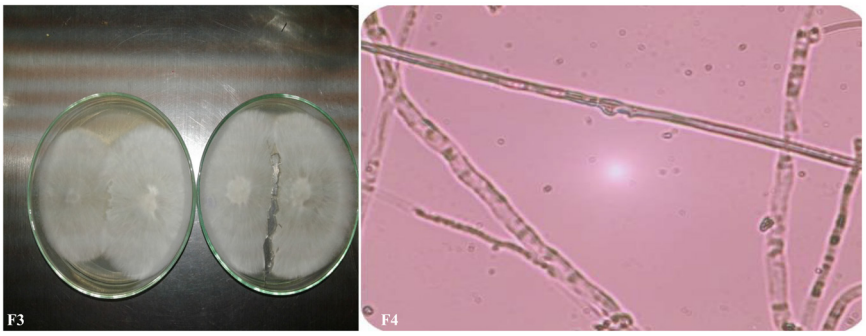

Fig. 3. Isolation of hybrids from meeting points. **Fig. 4.** Microscopy for presence of clamp connection and hyphal bridge to ascertain hybridization.

ed to study the qualitative and quantitative response during cultivation (Table 1).

Cultivation of hybrids : Jodon and Royse (1979) method was followed for making of spawn of hybrid cultures.

Preparation of mushroom cylinder : 2.5 kg sterilized paddy straw was filled from the opening side of polypropylene bag for about 3-4 inch 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 cylinder, 200 g 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 and by the insects.

Quantitative estimation of hybrid mushroom : 500 g freshly harvested fruit body mostly from the 1st flush were taken as a sample for calculating the dry matter. Freshly harvested mushroom was kept in hot air oven for drying at 60°C for 3 days and the individual dried sample were weighed for the calculation of dry weight of mushroom. Ashraf *et al.* (2013) has suggested one formula for calculating amount of moisture (%) percent of individual sample of mushroom.

$$\text{Moisture (\%)} = \frac{\text{Initial weight of sample —dried weight of sample}}{\text{Initial weight of sample}} \times 100$$

Most of the workers calculated biological efficiency as fresh weight of mushroom in relation to the dry weight of substrate but Cogorni *et al.* (2014) told 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 :

$$\text{Biological efficiency (\%)} = \frac{\text{Dry weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

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

RESULTS AND DISCUSSION

Spawn run and pinhead formation of 1st flush and 2nd flush

The earliness in fruiting of different interspecific hybrids was recorded by measuring days required for spawn run, pinhead formation and flushing of fruit bodies (Figs. 5—7). The earliness to reach different stages such as completion of spawn run in substrate, pinhead formation, production of 1st and 2nd harvestable fruit bodies within the interspecific hybrids were analyzed. The results showed that the spawn run complete was earliest in the hybrids namely PF × PSC5 and PF × PSC8 (20.67 days). This is followed by PF × PSC7 and PF × PSC1 (21 and 21.33 days, respectively). The duration of spawn run was significantly

Fig. 5. Spawn of PF × PSC hybrid. **Fig. 6.** Pinhead formation. **Fig. 7.** Fruit body production.

higher in the parents. This result is in accordance with El Kattan and Salama (1996) who reported that *P. florida* required 21 days for spawn run in rice straw and it decreased when straw was supplemented with legume waste. Pinhead formation was earliest in PF × PSC7 (3.33 days after spawn run) followed by PF × PSC4. In spite of early spawn run, PF × PSC3, PF × PSC5 and PF × PSC8 took quite longer duration for pinhead formation. The parent strains of *P. sajor-caju* although took longer period for spawn run but pinhead formation was earlier as compared to *P. flabellatus.*

Five hybrid combinations like PF × PSC12, PF × PSC14, PF × PSC16, PF × PSC17 and PF × PSC20 could not produce pinhead as there was no spawn run in those hybrids. This may be due to the fact these hybrids segregated in the 4th generation (spawn was from 3rd generation hybrids) and fruiting ability has been lost in them. Thus, these are aborted. Another interesting fact was noticed for PF × PSC11. In this combination, there was spawn run. But this hybrid even after full spawn run fails to produce pinhead i.e., fruiting body. This indicates the abortiveness of this hybrid at this stage. This may be due to the effect of reduced hybridization efficiency between them. These combinations were excluded from selection. Our

Table 2. Different parameters of different inter specific hybrids of *P. flabellatus* and *Pleurotus sajor-caju*.

ICRN	SR (days)	PH (days)	1st flush (days)	2nd flush (days)	3rd flush (days)	Fresh weight (g)	Dry weight (g)	BE (%)	Moisture (%)
PF × PSC1	21.33	4.67	3.33	6.33	10.33	812.33	81.97	8.19	89.90
PF × PSC2	27.33	5.00	2.67	5.00	0.00	407.67	41.26	6.18	59.93
PF × PSC3	21.67	9.33	4.67	10.00	10.00	1028.00	105.24	10.52	89.77
PF × PSC4	26.33	3.67	5.33	9.00	0.00	789.33	78.14	7.82	90.10
PF × PSC5	20.67	8.67	3.67	2.67	11.67	1270.00	129.43	12.93	89.81
PF × PSC6	29.33	4.33	3.67	5.67	0.00	1551.33	158.61	15.86	89.77
PF × PSC7	21.00	3.33	2.67	2.00	11.33	973.67	99.31	9.93	59.87
PF × PSC8	20.67	9.33	6.33	4.33	0.00	1276.67	130.54	13.05	89.77
PF × PSC9	29.67	4.33	2.33	6.33	0.00	521.33	47.17	4.71	60.63
PF × PSC10	28.00	5.33	3.33	8.33	15.67	1263.67	129.81	12.98	89.73
PF × PSC11	24.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PF × PSC13	37.33	6.33	6.00	10.67	0.00	433.00	37.27	3.72	91.43
PF × PSC15	31.00	4.67	8.33	0.00	0.00	353.33	27.04	2.70	92.47
PF × PSC18	38.33	7.33	3.67	0.00	0.00	564.33	57.28	5.72	89.89
PF × PSC19	26.00	8.00	5.67	3.33	10.33	561.67	51.52	5.15	90.78
PF control	23.00	5.67	4.67	7.33	0.00	722.00	70.65	7.06	90.22
PSC control	27.33	3.33	5.67	7.33	0.00	865.00	84.80	8.47	90.20
SEm±	2.664	1.007	0.741	1.141	1.918	174.91	17.388	1.665	12.634
CD (0.05)	7.657	2.893	2.130	3.278	5.513	502.696	49.973	4.785	36.311

result may be supported by the fact that in *Pleurotus*, among four basidiospores one type can mate only with one of the remaining three basidiospores, therefore the sterile : Fertile ratio is 75:25. It is only possible to get a clear picture of sterile: Fertile ratio, when the pairing will be maximum (Eger 1974, Liang and Chang 1989, Toyomasu and Morikan 1989).

ICRN : Interspecific cross number, SR: Duration of spawn run, PH: Duration from complete spawn run to pinhead formation, 1st F: Duration between pinhead formation to first flushes of fruit body, 2nd F: Duration between first flushes to second flushes of fruit body, Fwt: Fresh weight of total mushroom yield till 2nd flush, Dwt: Dry weight of total mushroom yield till 2nd flush, BE% : Biological efficiency, Moisture%: Moisture content in mushroom.

1st fruiting was found in PF × PSC9 (2.33 days) which is followed by PF × PSC2 and PF × PSC7 combination (2.67 days in both), whereas the 2nd fruiting body formation was earliest in PF × PSC7 (2 days) followed by PF × PSC5 (2.67 days). In some hybrids third flush was also noticed but those have taken a long time (10 or 11 days or even more than 15 days in 1 combination) as compared to first and second flush (Table 2).

PF × PSC7 completed very fast growth from spawning to second flush (only 29 days). So, even the yield of this hybrid is somewhat lower than the highest yielder, it can be recommended for cultivation for those farmers who require less time for their mushroom production. Since, the hybrids like PF × PSC7 and PF × PSC5 are producing very rapidly first and second flush, these can be advised to grow by the mushroom growers when high market demand is there. On the other hand, PF × PSC3, PF × PSC4 and PF × PSC10 took some long time from first flush to second flush. These hybrids are somewhat low yielder. But they can also be recommended for cultivation in the situation when less market demand for mushroom is there and farmer needs more time to dispose off the harvested mushrooms.

Fresh weight, dry weight and moisture% of interspecific hybrids : The results with respect to biological efficiency (BE), total dry and fresh weight of interspecific hybrids of *P. sajor-caju* and *P. flabellatus* showed that BE was highest in PF × PSC6 (15.8%) which signified the better ability of that hybrid to utilize the substrate. It was closely followed by PF × PSC8, PF × PSC10 and PF × PSC3 (13.05%, 12.98% and 12.93%, respectively). This result agrees with Baral *et al.* (2017) who found that one intra specific hybrid of *P. flabellatus* recorded biological efficiency of 11.74%. But our best hybrid produced more than that. It may be because inter specific hybridization produces more biological efficiency in mushrooms than intra specific hybrids.

Table 3. Nitrogen % and protein (mg/g) present in dry matter of hybrids.

Hybrids	N (%)	Protein (mg/g)
PF × PSC1	2.86	160.30
PF × PSC2	2.96	158.59
PF × PSC3	3.07	172.30
PF × PSC4	4.40	165.81
PF × PSC5	3.29	177.81
PF × PSC6	3.34	172.44
PF × PSC7	3.81	160.81
PF × PSC8	3.30	150.78
PF × PSC9	3.33	147.37
PF × PSC10	3.36	178.81
PF × PSC13	3.03	166.74
PF × PSC15	3.01	126.41
PF × PSC18	3.04	176.00
PF × PSC19	2.62	139.96
PF control	2.97	109.87
PSC control	2.87	147.25

Total fresh and dry weights of mushrooms were also significantly higher in those hybrid combinations. Production of fresh mushroom was recorded highest in PF × PSC6 (1551 g/2.5 kg of substrate) followed by PF × PSC8, PF × PSC5, PF × PSC10 (1276 g, 1270 g and 1268 g/2.5 kg of wet substrate, respectively). This result is similar with the findings of Kaur and Kapoor (2014) who reported that total fruit body yield of interspecific hybrids between *P. florida* and *P. sajor-caju* ranges normally from 507 g to 1156 g with 2 or 3 hybrids recording more than 2000 g yield. Corresponding dry weight of mushroom was also found significantly variable within the hybrids. The maximum dry weight of mushroom was recorded in PF × PSC6 (158.61 g/2.5 kg of substrate) and lowest in PF × PSC15 (27.04 g/2.5 kg of substrate) (Table 2).

Nitrogen % and protein % present in interspecific hybrids

Almost all the hybrids had showed the positive relation between the nitrogen and protein content. PF × PSC4 showed higher amount nitrogen (4.40%) which was closely followed by PF × PSC7 (3.81%), PF × PSC10 (3.36%), PF × PSC4 (3.33%). All the hybrids except PF × PSC1 and PF × PSC2 showed higher nitrogen content as compared to the parents (2.97%). PF × PSC1 and PF × PSC2 closely showed same amount of nitrogen as with the parents. Protein content was recorded highest in PF × PSC10 followed by PF × PSC5, PF × PSC6 and PF × PSC13 (178.8 -166.74 mg /g) of dry mushrooms (Table 3).

Most of the quality parameters of all our test inter specific hybrids of all the three combinations was in conformity with some workers. Baral *et al* .(2017) reported a maximum of 3.78% nitrogen in one intra specific cross between *P. flabellatus*. Pathania *et al.* (2017) recorded 3% nitrogen in one *Pleurotus ostreatus* in best supplemented treatment. Some of our hybrids are producing more than 4% nitrogen. So, it may be said that those hybrids became improved after inter specific hybridization.

Some hybrids recorded protein content of 160 to near about 180 mg/g of dry weight. It indicates that the protein content is 16 to 18% in those hybrids. These are very good amount of protein suggesting that these hybrids are good for human consumption as a protein source. Our result correlates the findings of Afiukwa *et al.* (2013) who found 16.35% protein content in *Pleurotus ostreatus*. The obtained values of protein in the mushrooms are compared well with 10.50% and 14.88% as reported by Okechukwu *et al.* (2011) for wood ear and oyster mushrooms, respectively in Owerri, Nigeria. These values are found to be low when compared with 18.07–37.00% reported for four species in India by Manjunathan *et al.* (2011). Our result also in conformity with El Kattan and Salama (1996) who recorded the highest value of protein in the range of 2.29 g/100 g fresh weight of *P. florida* when grown in rice straw supplemented with different materials. As we know that any composition in dry weight is 7 to 8 times more than its respective fresh weight. So, if we calculate then this protein value will become 16.03 to 18.32 g/100 g of dry mushroom weight. This finding is exactly similar with our finding (protein content 16 to 18 g/100g dry mushroom). Our value of protein is significantly higher than the findings of Gaur *et al.* (2016) who recorded protein content of 6.43 g/100g dry weight of *Pleurotus sajor- caju*. Since our experimental material is hybrid between two species, the higher value may be justified.

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