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Increasing the yield attributing character of different species of *Pleurotus* through hybridization

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ABSTRACT

Interspecific hybridization studies were carried out between *Pleurotus sajor-caju*, *P. sapidus* and *P. flabellatus* for obtaining better quality strains. Out of 48 crosses, only five inter specific crosses of *P. sajor-caju* x *P. sapidus* and four inters specific crosses of *P. sajor-caju* x *P. flabellatus* were compatible. Inter specific crossing between *P. sapidus* and *P. flabellatus* failed to show any compatible reaction. The compatible crosses were tested for evaluating their growth characteristics on MEA media and the cross SC2S1 have shown significantly higher mycelial growth rate (8.89 cm) which was followed by the cross SC1S1 (8.66 cm). The obtained hybrid crosses have shown more dense and regular growth with floccose, cottony and aerial mycelial texture and also showed off-white, pure white and yellowish white in colony colour. Out of nine dikaryotic strains, the cross SC2S1 (*P. sajor-caju* x *P. sapidus*) was the best strain among all the obtained hybrid strains and its parental strains in terms of number of days required for spawn run (11.50 days), number of days required for pin head formation (15.50 days), days required for harvesting (18.50 days), total number of fruiting body (214.00), weight of the individual fruiting body (20.00 g), total yield per bag (0.95 kg) and biological efficiency (95.00%). Whereas in terms of stipe diameter, the cross SC2F2 (*P. sajor-caju* x *P. flabellatus*) has shown maximum stipe diameter (3.50 cm) as compared to other dikaryotic strain and its parental strain and among the dikaryotic strain the cross SC1S2 (*P. sajor-caju* x *P. sapidus*) have shown significantly higher stipe length (6.41 cm), which was also higher than their respected parents. Maximum cap size (6.73 cm) was recorded in the cross SC1F2 (*P. sajor-caju* x *P. flabellatus*) which was significantly higher than the other dikaryotic strain and its parental strain. During sensory evaluations, the product B (*P. sajor-caju* x *P. flabellatus*) was rated better than the global mean in overall acceptance (8.56), taste (8.90), flavour (7.90), colour (7.40) and appearance (8.13), with highest score in all the sensory parameters followed by Product A (*P. sajor-caju* x *P. sapidus*). While the lowest score was obtained by *P. sapidus* (product D) followed by *P. flabellatus* (product E) and *P. sajor-caju* (product C).

Key words: Dikaryotic strains, Interspecific hybridization, *Pleurotus* spp.

Pleurotus which are white rot fungi of phylum basidiomycota having high saprophyte colonizing ability, can grow on wide 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. Oyster mushroom is one of the most commercially cultivated mushrooms in India and stands second in world production. The desirable attributes like rapid mycelia growth, high saprophytic

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colonization ability, simple and cheap cultivation techniques and easy post harvest storage have cultivated to popularity of *Pleurotus ostreatus* (Bhandal and Mehta, 1993). But inspite of easy method of cultivation the production of oyster mushroom is less in comparison to button mushroom in India and also cultivated mushrooms face problems like loss of genetic diversity and strain degeneration. Hence, there is an increased demand for the development of new and improved strains with high production and better growth attributes than their parents.

There are several methods for strain improvement in *Pleurotus* including selection, gene transformation and hybridization (Barh *et al.,* 2019). Understanding mushroom breeding systems is a major landmark when commercial breeding programs are being established (Larraya *et al.*, 2003). 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 Sharma, 2012; Jaswal *et al*., 2013). In *P. sajor-caju*, *P. florida* and *P. sapidus*, hybridization were carried out by Bahukhandi and Munjal (1990), Ghosh and Chakravarty (1991). Earlier, attempts have been made for producing quality strains of *Pleurotus*. In *P. ostreatus*, heterokaryons were developed by Eger *et al*. (1976).

MATERIALS AND METHODS

The laboratory experiments were done at Research Laboratory, Department of Plant Pathology, Assam Agriculture University, Assam, India. The culture media were sterilized in an autoclave at 15 p.s.i. for 15 minutes. Malt extract agar was the media used during the investigation. Fruit bodies from three Pleurotus species namely *P. sajor-caju*, *P.sapidus* and *P. flabellatus* were collected from the Lab of Department of Plant Pathology. Pure cultures were made from the stipe of fruit bodies by tissue culture technique. From the tip of hyphal growth mycelial segment was taken and transferred to Malt extract slants to get the pure culture of individual species. The mother cultures were kept at 4°C for further use.

Isolation of monosporus cultures

Isolation of monosporus culture is one of the most important steps to develop homokaryons before aiming of assembling new genes by dikaryotization in mushroom. Following two methods were followed to get the monosporus cultures of *P. sajor-caju, P.sapidus* and *P. flabellatus.*

i. **Spore print method:** Proposed by Petersen and Ridly (1996) was followed for the single spore isolation of *P. sajor-caju, P.sapidus* and *P. flabellatus*. The newly opened fleshy and healthy mushroom was selected to prepare a spore print. The cap of the mushroom was laid flat with gills down on a sterilized black paper which was placed inside a sterilized Petri dish. The Petri dish was sealed properly with paraffin and the entire setup was placed in an undisturbed area for 24 hours. The spores fell on the paper the next following day making a spore print pattern.

ii. **Serial dilution method:** The dilution method demonstrated by Bahukandi and Sharma (2012) was followed. Small loop of spore from the spore print was taken with the help of sterile needle and was suspended in sterile distilled water. From this suspension further dilution upto 10^{-4} was made, where the spore concentration was as low as up to 4-5 spores when seen under low power microscope (10x). Therefore from this solution a loop of 0.1 ml of spore suspension were carefully streaked on plain malt extract media in Petri plates and incubated at 25±1°C for germination. After 3-4 days of incubation, single spore isolates

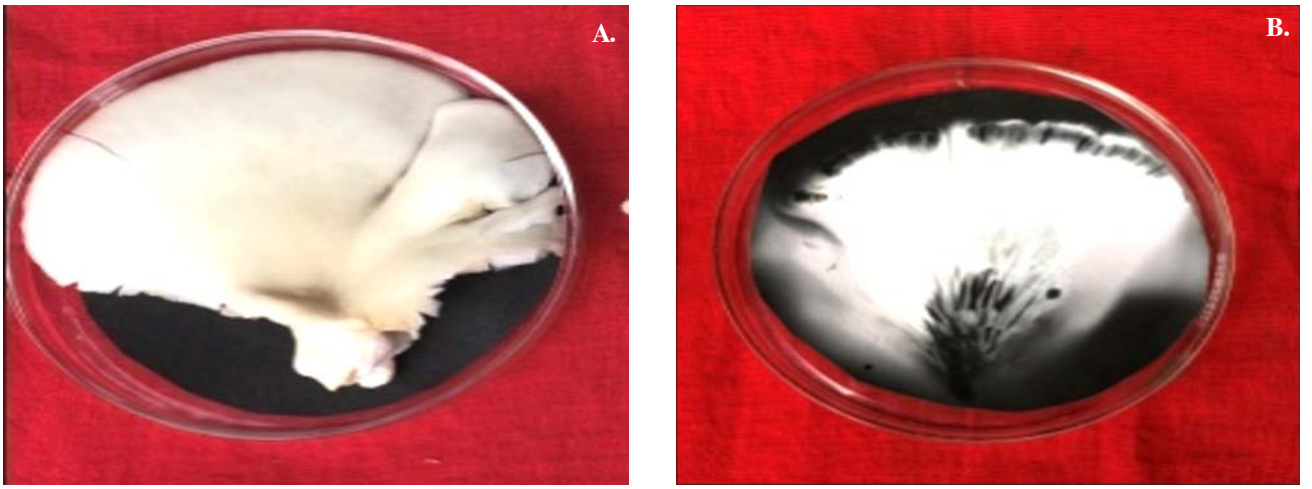


Fig. 1. Viable spores extracted from healthy and matured fruiting body, A. Discharging of spore from fruit body; B. Spore print from fruit body

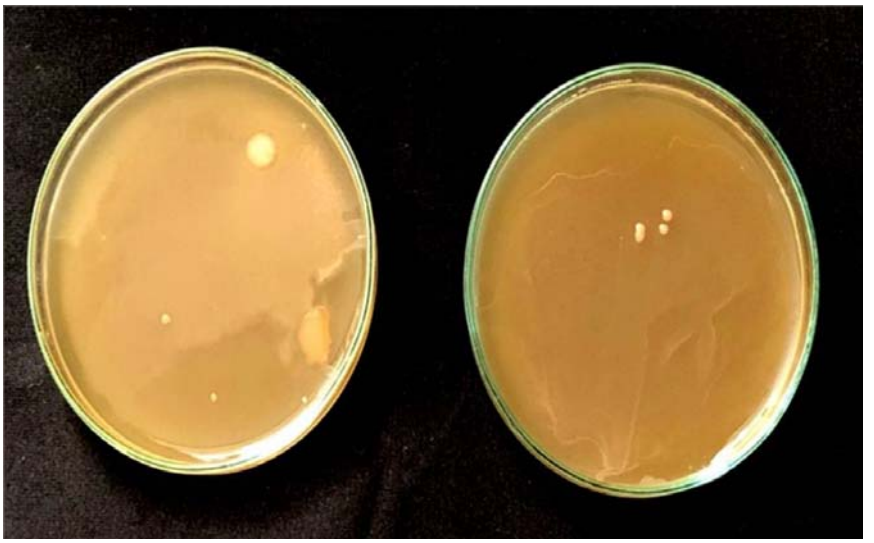


Fig. 2. Germinated single spores on MEA media

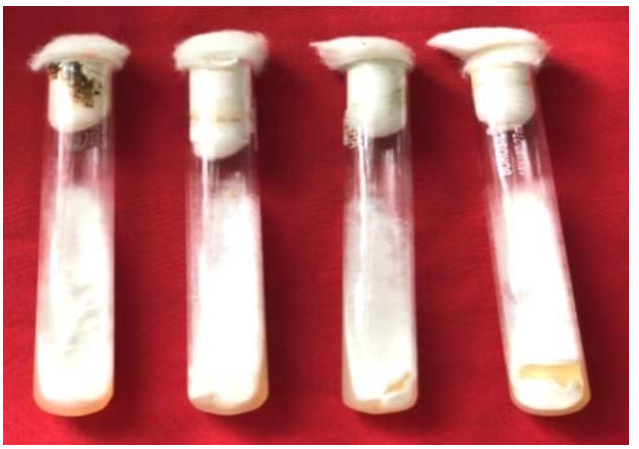


Fig. 3. Single spore germinated in MEA slants

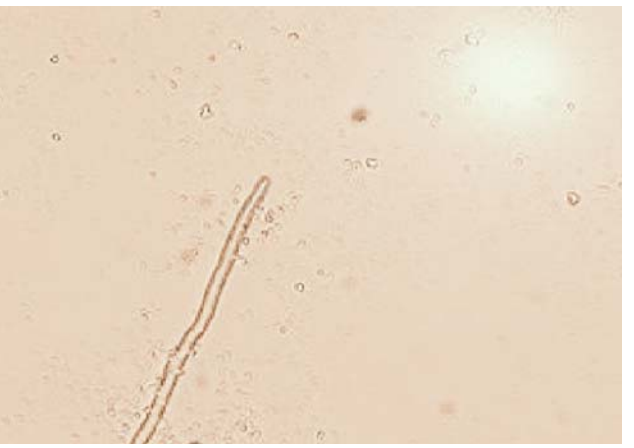


Fig. 4. Hyphal tip without clamp connection for isolation

(monokaryons) appeared as small colony heads which were transferred on to the malt extract slants and incubated for 25±1°C for 7-10 days. These monospore cultures were examined for the clamp connections. Those monospore cultures with no clamp connections were confirmed as monokaryons under the microcope at 45x and cultures showing presence of clamp connections (dikaryons) were discarded.

Inter-specific hybridization between three species: (Dual plate technique)

Four single spore cultures of each species *viz*. *P. sajorcaju, P. sapidus* and *P. flabellatus* were selected. 16 combinations of *P. sajor-caju* and *P. sapidus* followed by 16 combination of *P. sajor-caju* and *P. flabellatus* were made to test the compatibility as presented in the table 1 to 3. The crosses between monokaryotic cultures were performed by dual culture technique. Mycelial disc of three diameter from periphery of seven days old monosporus cultures of the above three studied species were cut with the help of sterile cork borer and were placed at 15 mm apart in the two opposite sides of the Petri plates containing malt extract agar medium order for the monokaryons to grow towards each other and the plates were incubated for about 7-10 days at 25±1°C. Depending on their compatibility, the dikaryons were picked, where the mycelium of the two species will show a dense aerial fluffy growth or barrage at the zone of confrontation. The crosses were considered positive when this cottony fluffy growth shows clamp connections which indicates dikaryotic nature of the mycelium when it was observed microscopically (45×). About 2mm strip of mycelium was taken by chopping from the meeting points of two different isolates and was carefully transferred to fresh medium for getting pure hybrid culture and stored for further mass culture preparation.

Table 1. Combinations of two monospores cultures of *P. sajor-caju* (SC) and *P. Sapidus* (S)

P. sajorcaju	***P. sapidus***			
	S_1	**S_2**	**S_3**	**S_4**
SC_1	SC_1S_1	SC_1S_2	SC_1S_3	SC_1S_4
SC_2	SC_2S_1	SC_2S_2	SC_2S_3	SC_2S_4
SC_3	SC_3S_1	SC_3S_2	SC_3S_3	SC_3S_4
SC_4	SC_4S_1	SC_4S_2	SC_4S_3	SC_4S_4

Table 2. Combinations of two monospores of *P. sajor-caju* (SC) and *P. flabellatus* (F)

P. sajorcaju	***P. flabellatus***			
	F1	**F2**	**F3**	**F4**
SC1	SC1F1	SC1F2	SC1F3	SC1F4
SC2	SC2F1	SC2F2	SC2F3	SC2F4
SC3	SC3F1	SC3F2	SC3F3	SC3F4
SC4	SC4F1	SC4F2	SC4F3	SC4F4

Table 3. Combinations of two monospores of *P. sapidus* (Sap) and *P. flabellatus* (F)

P. sajorcaju	***P. flabellatus***			
	F1	**F2**	**F3**	**F4**
SC1	S1F1	S1F2	S1F3	S1F4
SC2	S2F1	S2F2	S2F3	S2F4
SC3	S3F1	S3F2	S3F3	S3F4
SC4	S4F1	S4F2	S4F3	S4F4

In vitro evaluation of the performance of dikaryotic strains

After the confirmation of dikaryons, to screen out the fastest growing hybrid crosses the growth test was carried out in 9 cm Petri plates and small mycelial disc of about 5mm from the pure hybrid culture was transferred aseptically into fresh MEA media plates and were incubated for about 7-10 days at 25±1°C. After complete germination of the mycelia in the plates growth performance was recorded for the growth rate, colony morphology and colour.

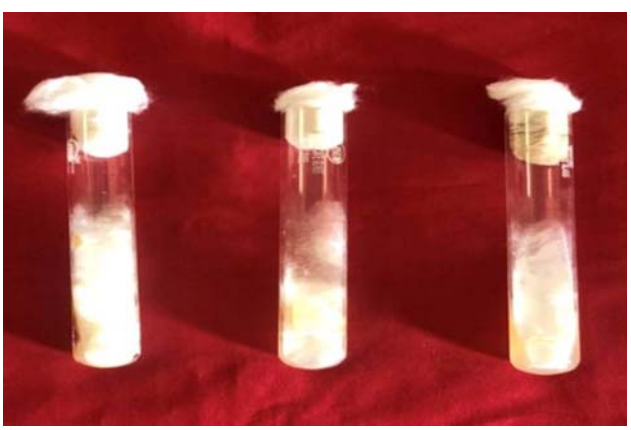
Preparation of mushroom bag

3kg sterilized rice straw was filled from the opening side of polypropylene bag for about 3 to 4inch

a. Dual Culture Plate

b. Dense aerial fluffy growth

c. Isolation of dikaryotic mycelium



d. Pure Hybrid culture

e. Mother spawn of obtained crosses

f. Inter specific crossing between *P. sapidus* and *P. flabellatus* failed to show any compatible reaction

Fig. 5. Steps in inter specific crossing in *Pleurotus* spp.

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.

Evaluation of yield of compatible crosses

The growth and development of mushroom were monitored daily. The time (number of days) required from inoculation to completion of mycelium running and time required from opening the plastic bags to first harvesting were recorded. Yield parameters such as total yield per bed and number of total fruiting bodies per bunch were observed and recorded. Growth parameters including stipe length (cm), stipe diameter (cm) and size of the cap (cm) were recorded after each harvest and colour of the matured fruit bodies were recorded too. Total weight of fruiting body was measured during each harvest and weighed using a weighing scale. Biological efficiency (B.E) was determined as per the formula (Chang, 1978) given below.

$$B.E\ (\%) = \frac{\text{Wt. of mushroom dy}}{\text{Dry wt. of straw}} X100$$

Sensory evaluation

Sensory quality of the obtained hybrid Mushroom along with the control *viz.*, *Pleurotus sapidus, Pleurotus sajorcaju* and *Pleurotus flabellatus* was evaluated by a sensory panel comprising of 15 member for colour, flavour, texture, taste and overall acceptability using a 9 point (1=dislike extremely, 9=like extremely) hedonic scale (Amerine *et al.*, 1965). Overall acceptability was determined by taking the average of colour, flavour, texture and taste score. Product characterization of hybrid strain and control species was obtained by using (XLSTAST ver. 2016.02.27444).

RESULTS AND DISCUSSION

Out of 48 crosses, only five inter specific crosses of *P. sajor-caju* × *P. sapidus* and four inters specific crosses of *P. sajor-caju* and *P. flabellatus* were compatible. Inter specific crossing between *P. sapidus* and *P. flabellatus* failed to show any compatible reaction. A total of nine compatible crosses were obtained and were subjected to fruiting trials. The compatible crosses were coded as SC1S1, SC1S2, SC2S1, SC2S3, SC3S1, SC1F1, SC1F2, SC2F2 and SC2F3.

In vitro mycelial growth performance of the dikaryotic strain in MEA media (cm)

Nine developed hybrids of *P. sajor-caju* × *P. sapidus* and *P. sajor-caju* × *P. flabellatus* were tested for evaluating their growth characteristics on MEA media. Monkaryotic strains of each three selected species were also tested for comparison with dikaryotic strain. The results presented in table 4 indicated that there is some variation in the mycelial growth rate of each dikaryotic strains with that of monokaryotic strains. It is clear from data that after

Table 4. *In vitro* mycelial growth performance of the dikaryotic strain in MEA media (cm)

Crossing Code	1 Days	2 Days	3 Days	5 Days	7 Days
SC1S1	0.93	1.660	4.522	8.750	8.660
SC1S2	0.89	1.475	4.150	8.400	8.085
SC2S1	0.97	1.922	4.907	9.320	8.987
SC2S3	0.84	1.782	4.125	8.225	8.37
SC3S1	0.86	1.567	4.200	8.200	8.562
SC1F1	0.93	1.637	4.422	8.697	8.727
SC1F2	0.73	1.480	4.247	7.937	7.771
SC2F2	0.72	1.407	4.125	8.150	7.860
SC2F3	0.83	1.335	4.250	8.175	7.971
P.SCm	0.51	1.252	3.300	7.617	7.685
P.SAPm	0.46	1.357	3.435	7.525	6.912
P.FLABm	0.65	1.342	3.442	8.125	7.152

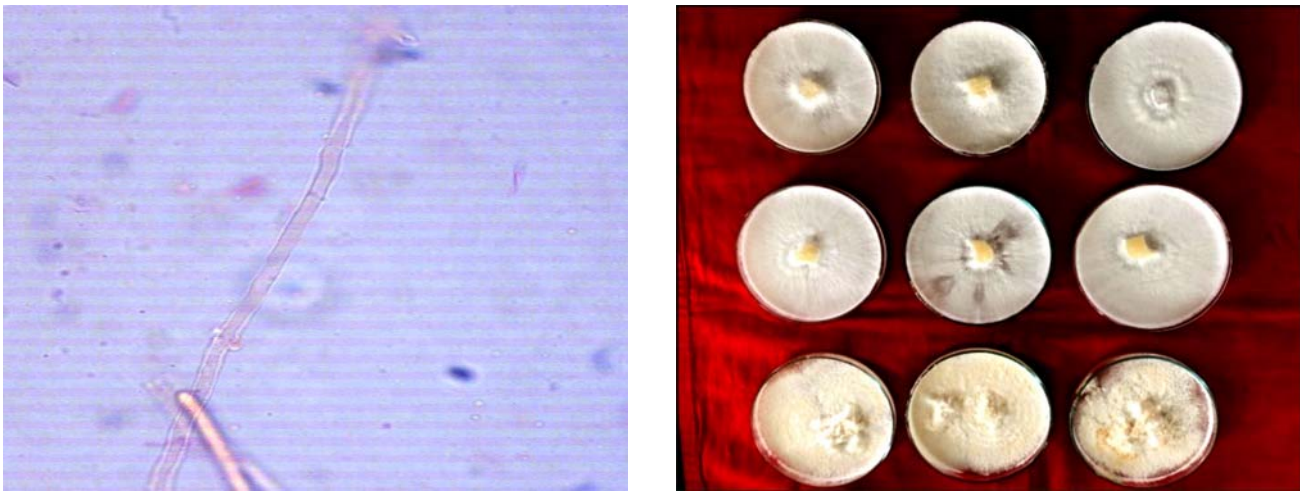


Fig. 6. Microscopy view for presence of clamp and colony morphology of the compatible connection to ascertain hybridization dikaryotic crosses

Spawn growth **Pin head formation**

Fig. 7. Evaluation of dikaryotic crosses in solid state fermentation

7 days most of the dikaryotic strains showed full mycelial growth in the Petri plates. Significantly higher mycelial growth was observed in the cross SC2S1 (8.97 cm) followed by SC1F1 (8.72) and among the dikaryons the lowest mycelial growth was observed in the cross SC1F2 (7.77 cm). The monokaryotic culture showed slower mycelial growth as compared to the dikaryotic strains and it took 8-10 days for full growth.

Evaluation of yield performance of compatible crosses

The earliness to reach different stages such as completion of spawn run in substrate, pinhead formation, harverting time, average stipe length, stipe diameter, cap size, cap diameter, total yield and biological efficiency within the inter-specific hybrids were calculated. Out of nine dikaryotic strains, we

Fig. 8. Fruit bodies production of different dikaryotic crosses

found that the cross SC2S1 (*P. sajor-caju* × *P. sapidus*) was the best strain among all the obtained hybrid strains and its parental strains in terms of number of days required for spawn run (11.50 days), number of days required for pin head formation (15.50 days), days required for harvesting (18.50 days), total number of fruiting body (214.00), weight of the individual fruiting body (20.00g), total yield per bag (0.95 kg) and biological efficiency (95.00%). Whereas in terms of stipe diameter, the cross SC2F2 (*P. sajor-caju* × *P. flabellatus*) has shown maximum stipe diameter (3.50 cm) as compared to other dikaryotic strain and its parental strain and among the dikaryotic strain the cross SC1S2 (*P. sajor-caju* × *P. sapidus*) showed significantly higher stipe length (6.41 cm), which was also higher than their respected parents. Maximum cap size (6.73 cm) was recorded in the cross SC1F2 (*P. sajor-caju* × *P. flabellatus*) which

was significantly higher than the other dikaryotic strain and its parental strain. The results indicated that there is no significant variation in terms of colour among the dikaryotic strains and their parents. The colours *viz*., white and greyish white were commonly observed.

These findings are supported by Bahukhandi and Sharma (2012) who carried out interspecific hybridization between *P. sajorcaju*, *P. sapidus* and *P. cornucopiae* to obtain better quality strains. A specific hybrid (hybrid no. 3) obtained by mating between *P. sajor-caju* and *P. cornucopiae*, in which the shape and size of the sporophore was similar to that of *P. sajor-caju* and white colour resembled with *P. cornucopiae*. The total average yield of this hybrid was found to be 23.3 per cent more than the parent. Kaur (2007) carried out intra-species crossing of *P. florida* and developed five *pleurotus* hybrid dikaryons among monokaryons of *P. florida* PAU-5, out of which PFJ 11 out yielded the parent strain. Spawn run was recorded faster in PFJ 11 (39 days) and PFJ14 (41 days) as compared to that of the parent (48 days). Ghosh and Chakravarty (1991) opined that in *Pleurotus sajor-caju* selective dikaryotization exert some exciting changes over the traditional cultures, improvement in quality characters, fast substrate colonising ability leading to early cropping and fruiting bodies with good size, shape and colour than their respected parents. Mating of inter-stock monokaryons in *P. ostreatus* has resulted in dikaryons with higher fruit body yield (Wang and Anderson, 1972).

Sensory evaluation

During sensory evaluation, *P. sajor-caju* × *P.flabellatus* (product B) was rated better than the

Table 5(a). Different growth parameters of the obtained hybrid strains and control species

Crossingcode	No. of days required spawn growth	No. of days required for pinhead formation	No. of days required for harvesting	Total no. of fruiting body/ bag	Averagestipe diameter (cm)	Average stipe length (cm)
SC1S1	12.00	17.50	20.00	210.25	1.85	4.97
SC1S2	13.75	18.50	22.25	160.50	2.73	6.41
SC2S1	11.50	15.50	18.50	214.00	2.35	4.60
SC2S3	15.00	19.25	22.50	139.00	3.14	5.29
SC3S1	13.00	17.50	21.75	206.75	2.47	5.10
SC1F1	12.50	17.75	21.00	208.25	2.92	5.99
SC1F2	16.75	22.25	25.25	161.25	2.89	5.24
SC2F2	16.00	20.75	25.00	167.50	3.50	4.69
SC2F3	14.00	18.50	22.00	201.25	3.16	6.27
P. sajorcaju	18.00	23.25	28.00	100.50	1.98	2.76
P. sapidus	18.75	22.75	29.25	105.25	1.33	2.45
P.flabellatus	17.00	29.75	33.00	121.50	1.67	3.33
SE(d)	0.71	0.784	1.04	8.061	0.44	0.35
$C.D_{0.05}$	1.44	1.596	2.13	16.41	0.91	0.73

SC1S1- *P.sajor-caju* × *P. sapidus*, SC1S2- *P.sajor-caju* × *P. sapidus*, SC2S1- *P.sajor-caju* × *P. sapidus,* SC2S3- *P.sajor-caju* × *P. sapidus,* SC3S1- *P.sajor-caju* × *P. sapidus,* SC1F1- *P.sajor-caju* × *P. flabellatus,* SC1F2- *P.sajor-caju* × *P. flabellatus,* SC2F2- *P.sajor-caju* × *P.flabellatus,* SC2F3- *P.sajor-caju* × *P. flabellatus.*

Table 5(b). Different growth parameters of the obtained hybrid strains and control species

Crossingcode	Average cap size (cm)	Weight of the individual fruiting body (g)	Total yield/bag of three harvest (kg)	Biological efficiency (%)
SC1S1	4.52	18.05	0.94	94
SC1S2	5.66	16.58	0.77	77
SC2S1	5.48	20.00	0.95	95
SC2S3	6.19	13.99	0.66	66
SC3S1	6.20	17.58	0.92	92
SC1F1	5.68	17.94	0.93	93
SC1F2	6.73	12.22	0.72	72
SC2F2	6.38	14.05	0.58	58
SC2F3	5.34	15.13	0.77	77
P. sajor-caju	5.99	9.95	0.54	54
P. sapidus	6.02	10.88	0.56	56
P.flabellatus	6.03	10.42	0.63	63
SE(d)	0.35	0.56	0.019	0.002
$C.D_{0.05}$	0.71	1.16	0.039	0.004

SC1S1- *P. sajor-caju* × *P. sapidus*, SC1S2- *P. sajor-caju* × *P. sapidus*, SC2S1- *P. sajor-caju* × *P. sapidus,* SC2S3-*P. sajor-caju* × *P. sapidus,* SC3S1-*P. sajor-caju* × *P. sapidus,* SC1F1-*P. sajor-caju* × *P. flabellatus,* SC1F2- *P. sajor-caju* × *P. flabellatus,* SC2F2-*P. sajor-caju* × *P.flabellatus,* SC2F3- *P. sajor-caju* × *P. flabellatus.*

global mean in overall acceptance (8.56), taste (8.90), flavour (7.90), colour (7.40) and appearance (8.13), with highest score in all the sensory parameters followed by *P. sajor-caju* × *P.sapidus* (product A). While the lowest score was obtained by *P.sapidus* (Product D) followed by *P.flabellatus* (product E) and *P.sajor-caju* (product C). Similar findings was given by Balakrishan (1994) where *P. sapidus, P. membranaceous* and *P. petaloides* obtained maximum consumer acceptability with respect to colour, appearance and flavor. Overall acceptability of this species was significant when compared to the standard species *P. sajor-caju* and *P. flabellatus* which were inferior in all the qualities.

CONCLUSIONS

From the present study, it can be concluded that *P. sajor-caju* × *P. sapidus* and *P. sajor-caju* × *P. flabellatus* were compatible and inter specific crossing between *P. sapidus* × *P. flabellatus* failed to show any compatible reaction. A total of nine compatible crosses were obtained and were subjected to fruiting trials. Among the dikaryotic strains the cross SC2S1 have shown significantly highest number (214 nos.) of fruiting body per bag which was followed by the cross SC1S (210 nos.) than their respected parental strains. All the dikaryotic strains showed higher yield attributing character than their respected parental strain. During sensory evaluations, *P. sajor-caju* × *P. flabellatus* (product B) was rated better with highest score in all the sensory parameters followed by *P. sajor-caju* × *P. sapidus* (Product A). While the lowest score was obtained by *P. sapidus* (product D) followed by *P. flabellatus* (product E) and *P. sajor-caju* (product C).

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