Interspecific hybridization in *Pleurotus* species

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ABSTRACT: Interspecific hybridization studies were carried out between *Pleurotus sajor-caju*, *P. sapidus* and *P. cornucopiae* for obtaining better quality strains. The resultant hybrids were categorised on phenotypic basis into two categories. In first group, hybrids obtained were similar in appearance, yield and colour etc. with either of their parents. The second group hybrids had blending of characters of their parents and possessing the yield potential more or less similar to them. A specific hybrid (hybrid no. 3) obtained with mating between *P. sajor-caju* and *P. cornucopiae*, in which the shape and size of the sporophore was similar to *P. sajor-caju* and white colour was resembling with *P. cornucopiae*. The total average yield of this hybrid was found to be 23.3 per cent more than the parent.

Key words: *Pleurotus*, oyster, interspecific hybridization, mating, desirable characters

Species of *Pleurotus* can grow on a number of substrates and a wide range of temperature. But inspite of easy method of cultivation its production is very less in comparison to button mushroom in India. *Pleurotus sajor-caju* is the most preferred species suiting to Indian conditions, having 22-28°C temperature range for its vegetative as well as reproductive growth. *Agaricus bisporus*, the common European white button mushroom requires more or less the same temperature requirement for its vegetative growth i.e. 22-25°C but prefers 16-18°C for its fruiting. In northern plains of India, growing season for both the mushrooms is more or less same, i.e. November to March for *Agaricus bisporus* and October to April for *Pleurotus sajor-caju*. As the button mushroom is preferred than the oyster mushroom due to socio-economic reason, the growers like to grow button mushroom during this season. In 1996-97, total mushroom production in India was 40,000 tons, of which *Agaricus* contributed 90 percent of the total production, whereas *Pleurotus* and *Volvariella* have only 10 percent share (Dhar, 1997). During rest of the year from May to September any of the two mushrooms (*Agaricus* and *Pleurotus*) can not be grown under natural conditions as the temperature during this period ranges between 28-46°C in northern plains. Therefore, there is a need to find out a strain which can be grown under natural conditions at a higher temperature and may have other desirable characters like white colour, sporelessness, better texture, taste and also high yielding capability. These qualities are not found in existing species of *Pleurotus*. The only exception is *P. fossilus*, which is still not cultivated commercially and brought from wild for consumption.

Earlier, attempts have been made for producing quality strains of *Pleurotus*. In *P. ostreatus*, heterokaryons were developed by Eugenio and Anderson (1968) and Eger *et al.*, (1976). In *P. sajor-caju*, *P. florida* and *P. sapidus*, hybridization were carried out by Bahukhandi and Munjal (1990), Ghosh and Chakravarty (1991), Thakur and Bhandal (1993). The hybrids obtained by these workers did not show much improved characters and hence lacked popularity and importance among mushroom growers. Therefore, the studies were carried out on interspecific hybridization among the selected three species of oyster mushrooms namely, *P. sajor-caju*, *P. sapidus* and *P. cornucopiae* due to their popularity in different parts of the world, to develop a suitable strain with desired characters.
MATERIALS AND METHODS

The type cultures of *P. sajor-caju*, *P. sapidus* and *P. cornucopiae* were obtained from Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi vide Acc. Nos. 1725, 2575 and 3312, respectively and sub-cultured at regular intervals. Spawn was made on wheat grains and the sporophores were obtained by standard cultivation method (Bahukhandi et al., 1989). The spore prints were taken from healthy mature sporophores, which served as spore stock for further study (Watling 1970).

Spore suspension was made by taking small loop of spores from the spore print and suspended in sterile distilled water. This solution was diluted to 10^4 dilutions, where spore concentration remains 4-5 spores per low power microscopic field (10X). One loop of this solution was streaked on plain agar in petriplates (pre-demarcated lines) and incubated at 25-27°C. The germinating spores were isolated and picked up by a needle/ spatula and placed in P.D.A. slants. A total of 26 single spore isolates of *P. sajor-caju*, 23 of *P. sapidus* and 19 of *P. cornucopiae* were obtained. These monokaryans were grown in mediated petriplates and the hybridization between the three species was done randomly. Three millimeter diameter bits were cut out from seven days old monokaryotic culture and the two monokaryotic cultures were placed at two opposite ends in petriplates containing PDA. The single spore isolates were kept for interspecific crossing in the following way (Table 1).

1. *P. sajor-caju* x *P. sapidus* = 26x23=598 pairings
2. *P. sajor-caju* x *P. cornucopiae* = 26x19=494 pairings
3. *P. sapidus* x *P. cornucopiae* = 23x19=437 pairings

In this way a total of 1519 number of pairings were obtained. These paired isolates were incubated at 25-27°C for 7-10 days for mating. A small inoculum was taken from the meeting point of the paired monokaryons if any. The spawn was made by the standard method and cultivation was done by the method described earlier (Bahukhandi et al., 1989). The sporophore characters like colour, size, yield and temperature requirement for cultivation were recorded in different hybrids and compared with their parents. Hybrids were grown on chopped paddy straw in small polybags and were categorized in 9 groups based on size, shape, colour and yield etc. From each group one type sample having higher yield, early fruiting and light-colour was selected and were used for further cultivation studies. Hybrid nos. 1-3 were as a result of mating between *P. sajor-caju* x *P. cornucopiae*, 4-6, were between *P. sajor-caju* x *P. sapidus* and 7-9 were between *P. sapidus* x *P. cornucopiae*. Cultivation studies were carried out in polybags having capacity of one kg. dry straw. Time taken for substrate colonization, cropping period and total yield obtained in 4 flushes was recorded.

The non-mated pairs in all the three crossing (*P. sajor-caju* x *P. sapidus*, *P. sajor-caju* x *P. cornucopiae* and *P. sapidus* x *P. cornucopiae*) were categorized into 3 classes namely a, b and c according to their hyphal growth pattern.

| Table 1. Number and type of mated and non-mated pairs and hybrids obtained after mating three species of *Pleurotus* |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Species of *Pleurotus* | Total Pairs obtained | Total mating pairs | Segregation of Hybrids (fertile) | Total non mating pairs | Morphological variations (sterile) |
|-----------------|----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| *P. sajor-caju* x *P. sapidus* | 598 | 92 | 26 | 506 | 425 | 58 | 23 |
| *P. sajor-caju* x *P. cornucopiae* | 494 | 80 | 23 | 414 | 360 | 42 | 12 |
| *P. sapidus* x *P. cornucopiae* | 437 | 90 | 28 | 347 | 308 | 23 | 16 |

A-Hybrids with characters of first-parent, B-Hybrids with blending of characters of both the parents, C-Hybrids with characters of second parent, a-Simple non mated pairs, b-Neutral, c-Inhibitory
RESULTS AND DISCUSSION

It was observed that the single spore isolates obtained in the three species of *Pleurotus* have some peculiar features, by which they were differentiated from dikaryotic mycelium, (i) The single spore isolates were having slower growth and in some cases the growth was limited to inoculum. (ii) Absence of clamps. (iii) They did not produce fruiting bodies, during cultivation. Although some primordial formation were seen in some monokaryons but they were abortive and did not grow further. Some of the isolates were not able to colonize the substrate (Fig. 1).

During the mating process between monokaryons, four types of reactions were seen in petriplates on the basis of the nature of the growth of hyphae. As a result of first reaction mating occurred and fertile progeny was produced (A, B and C, Table 1). In this process the hyphae developing from both the oppositely placed inocula were anastomosed and showed clear difference in appearance between individual parents and mating hyphae, when tested for fertility, they were found to be fertile (Fig. 2).

The non-mated pairs belonging to remaining three categories have some special morphological characters (a,b and c, Table 1). In first category (a), the hyphae of the monosporous pairs were intermingled and gave an idea that these have mated, but after testing they did not produce fruit bodies and were found sterile. Most of the non mated pairs belonging to this category (425, 360 and 308 respectively in the 3 crosses). In the second category the hyphae of both the paired monospores grew but did not mate and remain separated. This reaction of the non mated isolates was neutral (b) and their number in each crossing were 58, 42 and 23 respectively. In the third category, a zone of inhibition was developed between paired isolates and their growth occurred in different direction to each. This reaction was termed as inhibitory (c) and the number of pairs in this category were lowest among all the three crosses (23, 12 and 16 respectively).

In fertile isolates (first category) dikaryotization, clamp connection and sporophore formation were observed. In three sets of possible crosses the sterile:fertile ratios were recorded as 506:92, 414:80 and 347:90, respectively in *P. sajor-caju* x *P. sapidus*, *P. sajor-caju* x *P. cornucopiae* and *P. sapidus* x *P. cornucopiae*. The hybrids obtained in the present study in all the above pairings showed characters corresponding to their parents. There was hardly any difference between hybrids and their parents in their vegetative growth characters. The sporophores in all the above combinations were categorized in two types. Some of the hybrids were similar to their either of the parents and some of them had blending of characters from

![Fig.1. Mycelial growth of a dikaryon (A) and monokaryon (B) of *Pleurotus sajor-caju*](image)
both the parents. In first set of mating \((P.\, sajor-caju \times P.\, sapidus)\) 26 hybrids (sample hybrid-4) were phenotypically similar to \(P.\, sajor-caju\) (Fig. 3A) and 21 (sample hybrid-5) were similar to \(P.\, sapidus\), (Table 1, Fig. 3C). Remaining 45 hybrids (sample hybrid-6) were possessing blending of characters between the two parent (Fig. 3B). The similarities were considered in appearance, colour, size and texture of fruiting bodies and temperature requirements for cultivation of the hybrids, etc. The hybrids had blending of characters having colour of sporocarp like \(P.\, sapidus\) and size like \(P.\, sajor-caju\) but there was no other specific character and the yield was also similar to their parents (Table 2 shows sample hybrids selected for cultivation as 4, 5, 6 nos.)

In the crosses between \(P.\, sapidus \times P.\, cornucopae\), more or less same results were obtained and the resultant number of three types of hybrids were 28:40:22 (Table 1, Fig 3). The hybrids did not show any specific character and were similar in all the respects including yield, with their parents (Table 2 shows sample hybrids selected for cultivation as 7, 8, 9 respectively).

In the third set of crossing between \(P.\, sajor-caju \times P.\, cornucopae\), the phenotypic ratio of hybrids was 23:39:18 (Table 1, Fig. 3D,E). All hybrids performed more or less similar results as in first two cases (Table 2 shows sample hybrids A, C and D selected for cultivation as 1, 2, 3 respectively). The authors took only sample hybrids from each groups obtained after hybridization among the three species numbered as 1, 2, 3, 4, 5, 6, 7, 8 and 9 as mentioned in the text. It has been observed that total yield in all the hybrids

<table>
<thead>
<tr>
<th>Parents and Hybrids</th>
<th>Time for substrate colonization (days)</th>
<th>Cropping period after colonizationflushes (days)</th>
<th>Yield for 4 flushes (g)</th>
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<tr>
<td>Parents</td>
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<tr>
<td>(P., sajor-caju)</td>
<td>16</td>
<td>38</td>
<td>900</td>
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<tr>
<td>(P., sapidus)</td>
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<td>40</td>
<td>840</td>
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<tr>
<td>(P., cornucopae)</td>
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<td>42</td>
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<td>1080</td>
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<td>Hy. 8</td>
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<td>800</td>
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<tr>
<td>Hy. 9</td>
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<td>38</td>
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*Hybrid No. 3 deposited vide I.T.C.C. Acc. No. 3828 in Indian Type Culture Collection
was more or less similar, however hybrid no. 3 (Fig. 3E) as a result of mating between P. sajor-caju and P. cornucopiae, took lesser time in substrate colonization and higher yield in shorter cropping period than rest of the isolates (Table 2). The fruit bodies of hybrid no. 3 were of whitish colour, which is an additional advantage over others as people prefer white-coloured mushrooms. It was also found to produce fruit bodies up to 32°C.

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![Image](image_url)

**Fig.3.** P. sajor-caju, P. sapidus and P. cornucopiae and inter-specific hybrids developed by crossing among them. A. Hybrids resembling to P. sajor-caju, B. Hybrids having blending of characters of P. sajor-caju and P. sapidus, C. Hybrids resembling to P. sapidus, D. Hybrids having blending of characters of P. sajor-caju and P. cornucopiae, E. Hybrids no. 3 recommended for cultivation.

The data reveals that the hybrid no. 3 is better yielder than the others and may be recommended for cultivation to the mushroom growers. The culture of hybrid no.3 was deposited in Indian Type Culture Collection, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi vide Acc. No. 3828 (Table 2).

Pleurotus is heterothallic and tetrapolar (Anderson et al., 1973, Bahukhandi and Munjal 1990, Thakur and Bhandal 1993). A single basidiocarp produces four types of basidiospores with two different mating factors termed as ‘A’ and ‘B’. Dikaryon is produced by the basidiospores having different incompatibility factors, which can be detected on the basis of presence of clamps and conjugate nuclear division (Raper, 1966). Roxon and Jong (1977) described four mating types of Pleurotus sajor-caju as A1B1, A2B2, A1B2 and A2B1. Kaufert (1936) reported four types of reactions, when he mated monokaryons of P. corticus and observed mated or compatible, neutral, antagonistic and inhibitory reactions. In the present study, however the paired monokaryons resulted in three types of colonies, which may be termed as mated, non mated, neutral and antagonistic. This combination may be compared with the results of Kaufert (1936). It is also reported that interstock hybrids gave better yield than intrastock hybrids in Pleurotus species (Jandaik 1987, Rajaratnam and Bano 1987, Thakur and Bhandal 1993). During this study it has been observed that the resultant hybrids produced more or less similar yield as their parents but the hybrid produced by crossing between P. sajor-caju and P. cornucopiae, resulted 23.3 per cent more yield as compared to his parents, which is in agreement with that of earlier workers. Like Schizophyllum and Coprinus, in Pleurotus also, 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 Mori 1989).

In the present study, the F1 hybrid (Hybrid No. 3) obtained by crossing between P. sajor-caju and P. cornucopiae, having blended characters but higher yield than its parents, while other F1-hybrids were almost similar to their parents in terms of yield potential and other morphological features. Further studies in this direction are required for obtaining other desirable traits in the new hybrids.

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REFERENCES


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