DEVELOPMENT OF IMPROVED STRAIN IN OYSTER MUSHROOM (*Pleurotus* spp.)

by

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(2017-11-060)

THESIS

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2019

DECLARATION

I, hereby declare that this thesis entitled **"Development of improved strain in Oyster mushroom (*Pleurotus* spp.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CONTENTS

Sl. No.	Chapter	Page No.
1	INTRODUCTION	1-2
2	REVIEW OF LITERATURE	3-24
3	MATERIALS AND METHODS	25-38
4	RESULTS	39-65
5	DISCUSSION	66-82
6	SUMMARY	83-87
7	REFERENCES	88-105
	APPENDICES	106-114
	ABSTRACT	115-117

LIST OF TABLES

Table No.	Title	Page No.
1	Biometric observations of sporocarp of species of *Pleurotus* cultivated in paddy straw substrate	41
2	Microscopical observations of species of *Pleurotus* cultivated in paddy straw substrate	41
3	Cultural characters and mycelial growth rate of species of *Pleurotus* in PDPA medium	43
4	Comparative performance of species of *Pleurotus* and their mixed spawn cultivated in paddy straw substrate	45
5	Comparative performance of species of *Pleurotus* and their hybrids developed through hybridization followed by three generation of selection in paddy straw substrate	48
6	Biometric observations of sporocarp of species of *Pleurotus* and their hybrids cultivated in paddy straw substrate	50
7	Comparative performance of *P. djamor* and their gamma irradiated (20 and 25 Gy) mutants developed through gamma irradiation followed by three generation of selection in paddy straw substrate	52
8	Comparative performance of *P. florida* and their gamma irradiated (20 and 25 Gy) mutants developed through gamma irradiation followed by three generation of selection in paddy straw substrate	53
9	Comparative performance of *P. ostreatus* and their gamma irradiated (20 and 25 Gy) mutants developed through gamma irradiation followed by three generation of selection in paddy straw substrate	54
10	Comparative performance of species of *Pleurotus* and their gamma irradiated (20 and 25 Gy) mutants developed through gamma irradiation followed by three generation of selection	55
11	Comparative performance of species of *Pleurotus* and their improved strains developed through hybridisation and gamma irradiation followed by three generation of selection	57
12	Analysis of proximate contents species of *Pleurotus* and their improved strains developed through hybridisation and gamma irradiation followed by three generation of selection	59

13	RAPD scoring pattern of species of *Pleurotus* and their hybrids	64
14	Similarity matrix for Jaccard's coefficient of species of *Pleurotus* and their hybrids	64
15	RAPD scoring pattern of species of *Pleurotus* and their gamma irradiated mutants	65
16	Distance matrix for Jaccard's coefficient of species of *Pleurotus* and their gamma irradiated mutants	65

LIST OF PLATES

Plate No.	Title	Page No.
1	Sporocarp of *P. djamor* (pink and spathulate shaped pileus with short stipe)	41-42
2	Development stages of *P. djamor* from pinheads to harvesting maturity	41-42
3	Sporocarp of *P. florida* (white and spathulate shaped pileus with stout stipe)	41-42
4	Development stages of *P. florida* from pinheads to harvesting maturity	41-42
5	Sporocarp of *P. ostreatus* (greyish white pileus with long stipe and falcate decurrent gills)	41-42
6	Development stages of *P. ostreatus* from pinheads to harvesting maturity	41-42
7	Mycelium of species of *Pleurotus* with clamp connections	41-42
8	Clavate shaped tetrasterigmatic basidia of species of bearing basidiospores	41-42
9	Cylindrical shaped basidiospores of species of *Pleurotus*	41-42
10	Subventricose to clavate shaped cystidia of species of *Pleurotus*	41-42
11	Spore print of species of *Pleurotus* indicating their gill arrangements	41-42
12	Radial growth of species of *Pleurotus* in PDPA medium on 9th day of inoculation	43-44
13	Cultural characters of species of *Pleurotus* in PDPA medium	43-44
14	Production of fruiting bodies of both *P. djamor* and *P. florida* on their spawn mixed bed	45-46
15	Reduced yield performance and crop period of mixed spawn of *P. djamor* and *P. florida* at 40 days after spawning compared to *P. djamor* and *P. florida*	45-46
16	Production of fruiting bodies of both *P. djamor* and *P. ostreatus* on their spawn mixed bed	45-46

17	Reduced yield performance and crop period of mixed spawn of *P. djamor* and *P. ostreatus* at 40 days after spawning compared to *P. djamor* and *P. ostreatus*	45-46
18	Single spore culture of *P. djamor, P. florida* and their dual culture	46-47
19	Single spore culture of *P. djamor, P. ostreatus* and their dual culture	46-47
20	Mycelium of single spore culture of species of *Pleurotus* and their hybrids indicating absence/ presence of clamp connection	46-47
21	Colony morphology of species of *Pleurotus* and their hybrids in PDPA medium	46-47
22	Earliness in spawn production of hybrids compared to their parental species of *Pleurotus* on 9th day of culture inoculation	48-49
23	Earliness in primordial initiation in hybrids compared to their parental species of *Pleurotus* on 12th day after spawning	48-49
24	Enhanced yield in hybrid of *P. djamor* and *P. florida* compared to *P. djamor*	48-49
25	Enhanced yield in hybrid of *P. djamor* and *P. ostreatus* compared to *P. djamor*	48-49
26	Blended morphological characters of species of *Pleurotus* in their hybrids	55-56
27	Earliness in primordial initiation in *P. djamor* gamma irradiated at 20 Gy on 13th day after spawning compared to *P. djamor* and *P. djamor* gamma irradiated at 25 Gy	55-56
28	Enhanced yield in *P. djamor* gamma irradiated at 20 Gy compared to *P. djamor* and *P. djamor* gamma irradiated at 25 Gy	55-56
29	Larger sporocarps of *P. florida* gamma irradiated at 25 Gy compared to *P. florida* and *P. florida* gamma irradiated at 20 Gy	55-56
30	Earliness in primordial initiation in *P. ostreatus* gamma irradiated at 25 Gy on 20th day after spawning compared to *P. ostreatus* and *P. ostreatus* gamma irradiated at 20 Gy	55-56
31	Enhanced yield in *P. ostreatus* gamma irradiated at 25 Gy compared to *P. ostreatus* and *P. ostreatus* gamma irradiated at 20 Gy	55-56

32	Enhanced yield in selected strains of species of *Pleurotus* developed through hybridisation and gamma irradiation followed by three generation of studies compared to parents	57-58
33	Fungal contaminants observed in mushroom beds of species of *Pleurotus*	60-62
34	Pests observed in mushroom beds of species of *Pleurotus*	60-62
35	RAPD profile of species of *Pleurotus* and their hybrids amplified by primer OPT 5 (GGGTTTGGCA)	65-66
36	RAPD profile of species of *Pleurotus* and their gamma irradiated mutants amplified by primer OPS 5 (TTTGGGGCCT)	65-66

LIST OF FIGURES

Fig No.	Title	Page No.
1	Steps for preparation of mixed spawn of species of *Pleurotus*	29-30
2	Steps for hybridization by crossing single spore culture of species of *Pleurotus*	31-32
3	Steps for improving the strain by gamma irradiation of species of *Pleurotus*	32-33
4	Comparison of days required for primordial initiation in species of *Pleurotus* and their mixed spawn	69-70
5	Yield comparison of species of *Pleurotus* and their mixed spawn	69-70
6	Comparison of days required for primordial initiation in species of *Pleurotus* and their hybrids	73-74
7	Yield comparison of species of *Pleurotus* and their hybrids	73-74
8	Comparison of days required for primordial initiation in species of *Pleurotus* and their gamma irradiated (20 and 25 Gy) mutants	75-76
9	Yield comparison of species of *Pleurotus* and their gamma irradiated (20 and 25 Gy) mutants	75-76
10	Comparison of moisture (fresh weight), carbohydrate (dry weight), protein (dry weight) and fibre content (dry weight) in species of *Pleurotus* and their improved strains developed through hybridization and gamma irradiation followed by three generation of selection	78-79
11	Dendrogram of species of *Pleurotus* and their hybrids constructed by UPGMA of binary matrix obtained from RAPD	80-81

LIST OF APPENDICES

Sl. No.	Title	Page No.
1	Data sheet	106
2	Composition of stain used	111
3	Composition of media	112
4	Weather data at AAS, Vellayani, KAU	113
5	Fasta sequence of isolate 3 (*P. ostreatus*)	114

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
µg	Microgram
µL	Microlitre
µm	Micrometer
^{0}C	Degree Celsius
ANOVA	Analysis of variance
BE	Biological efficiency
bp	base pair
CD	Critical difference
CEA	Carrot Extract Agar
cm	Centimetre
CRD	Completely Randomised Design
Cs	Caesium
DNA	Deoxyribo nucleic acid
et al.	And other co-workers
Fig.	Figure
g	Gram
Gy	Gray
h	Hour
KAU	Kerala Agricultural University
kb	Kilobase pair
kg	Kilogram
kGy	Kilogray
kr	Kilorad
L	Litre
MEA	Malt Extract Agar
mg	Milligram
min.	Minute
mL	Millilitre

mm	Millimeter
mM	Millimolar
N	Normality
ng	Nanogram
OMA	Oat Meal extract Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDPA	Potato Dextrose Peptone Agar
pmol	Picomole
PYDA	Potato Yeast Dextrose Agar
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RH	Relative humidity
rpm	Rotations per minute
SE (m) $\pm$	Standard error of mean
SD	Standard deviation
sec	Second
Sl.	Serial
sp. or spp.	Species (Singular and plural)
temp.	Temperature
UPGMA	Unweighted pair group method with arithmetic mean
V	Voltage
viz.	Namely
WEA	Wheat Extract Agar
YEA	Yeast Extract Agar

Introduction

1. INTRODUCTION

Mushroom is a macrofungus with a distinctive fruiting body which are large enough to be seen with naked eye and picked by hand. Cultivation of mushroom has a long history and over twenty species are commercially cultivated. Among edible mushrooms, *Pleurotus* species stands second in world production with 19 per cent contribution to total mushroom production (Sharma *et al.,* 2017).

Oyster mushroom (*Pleurotus* spp.) is commonly called as Dhingri in India because of its oyster like shape. The genus has more than 50 well recognized species out of which 25 have been reported from India and 12 are cultivated in different parts of the country. Different species of *Pleurotus* grow well in wide temperature and thus suited for year around cultivation in various regions of tropical country like India.

Owing to their favourable organoleptic, neutraceutical and therapeutic properties, its popularity is increasing worldwide, particularly in Asia and Europe. In addition to this, they have high biological efficiency (BE) and significant levels of proteins (Kalmis *et al.*, 2008), while low in fats, calories, sodium, carbohydrates and cholesterol (Gupta *et al.*, 2011).

Oyster mushroom is the well accepted one in Kerala, for its broad adaptability under diverse agro-climatic conditions. There are 15 to 20 species of oyster mushrooms, reported from Kerala. Moreover, the tropical climate of Kerala makes it possible to commercially cultivate Pleurotus in a wide variety of substrates. Thus there are great opportunities in this field which can lead to enterprise diversification and income generation.

High production and productivity, along with good quality are always the principal goals for agriculturally important crops and the mushrooms are not exceptional. The cultivated mushrooms face the problems like loss of genetic diversity and strain degeneration (Wang *et al.*, 2012). Another hurdle in the commercial cultivation is that, many high yielding mushrooms have longer

duration whereas certain others have short duration with low yield. Much of the work has been done on its cultivation and little attempt has been made to find genetic variability of species. Thus it is prerequisite to explore and collect diverse germplasm to develop strains with improved characteristics to make mushroom cultivation sustainable and highly productive.

Under the above scenario, there is a need on development of improved strain in *Pleurotus* spp. to ensure both cultivator and consumer preferences. For the study, three isolates of *Pleurotus* spp., *Pleurotus djamor* (isolate 1), *Pleurotus florida* (isolate 2) and a native isolate with 80 per cent similarity to *P. florida* (isolate 3), which have wide acceptability in Kerala, were selected. Among the available isolates, *P. florida* was a well-known mushroom which needed maximum days (22 days) for primordial initiation and had 80.08 per cent BE (Chauhan, 2013). The other native isolate collected also had long duration for primordial initiation like *P. florida*, but with higher BE. Whereas *P. djamor* had comparatively minimum days (16 days) for primordial initiation and yielded low (430.5 g kg^{-1} substrate) with fibrous fruiting bodies in paddy straw (Satpal *et al.*, 2017; Jose, 2018). The leathery texture of isolate 1 though favours good transit and packaging, it has less consumer preference compared to isolate 2 and isolate 3, which is having delicate texture.

Strains could be improved through various methods like hybridisation by spawn mixing, hybridisation by crossing single spores and gamma irradiation (Anitha, 1998). Hybridisation could induce variability in the existing germplasm by combining desirable characteristics from different isolates of *Pleurotus* spp. Whereas, gamma irradiation induced mutation is one of the convenient methods for the development of new strains with better quality traits and productivity. Hence the present study aims at developing an improved strain of *Pleurotus* sp. with high BE and short duration from the native isolates by hybridisation and gamma irradiation, which in turn increase farmers' revenue.

Review of Literature

2. REVIEW OF LITERATURE

The genus *Pleurotus* commonly known as oyster mushroom comprises more than 50 well recognised species and is distributed worldwide. *Pleurotus* spp. is second position in world production (19%) following Shiitake (22%) mushroom (Sharma *et al.*, 2017).

Pleurotus spp. were well appreciated for their neutraceutical properties like protein, fibre, carbohydrates, vitamins and minerals (Menaga *et al.*, 2012). Owing to their high nutritional and medicinal property, demand for the same is increasing. There were around 15 to 20 *Pleurotus* spp. available for cultivation in Kerala (Krishnapriya, 2018), of which *P. florida, P. eous, P. djamor, P. sajor-caju* and *P. citrinopileatus* has gained high popularity.

2.1 MORPHOLOGICAL CHARACTERISATION OF SPOROCARP OF NATIVE ISOLATES

Stamets (2000) reported that *Pleurotus* spp. was known for its shorter duration, ability to flourish on wide variety of substrates and temperature. It preferred tropical and subtropical climate for better performance.

In India, *P. djamor* (Fr.) Boedijin a new edible species from Tamil Nadu was first reported by Geetha and Sivaprakasam (1993). *P. djamor* was also identified from forest regions of Shimoga and Kodagu of Karnataka (Pandey and Veena, 2012), tropical moist deciduous forests of Tripura (Das *et al.*, 2014) and dried acacia tree logs in North Bengal (Roy *et al.*, 2015). Karun and Sridhar (2016) identified *P. djamor* from Western Ghat forest of Karnataka.

Bernardo *et al.* (2004) studied the macro and micromorphological characters of the genus Pleurotus obtained from field and different national herbaria in Argentina. Based on morphological features, these were identified as *P. albidus*, *P. cystidiosus*, *P. ostreatus*, *P. pulmonarius*, *P. rickii* and *P. djamor*.

2.1.1 Macroscopical Observations

P. djamor var. *roseus*, was also called as pink oyster mushroom, as it produced pinkish red coloured sporocarp. It was characterized by spathulate to flabelliform pileus, lateral short stipe, dimitic hyphal system and leathery nature (Corner, 1981). Periasamy and Natarajan (2003) reported the margin of wild and recombinant strains of *P. djamor* as entire, wavy, petaloid and multiwavy. Junior *et al.* (2010) reported that *P. djamor* (Rumph. ex Fr.) Boedijn, had a pileus dimension of 70-100 mm × 55-85 mm, with pinkish colour when fresh, and turning cream or yellowish when dry. The surface and margin was smooth. Stipe was usually absent or reduced with 5-10 mm length and 4-8 mm stipe width. The stipe was attached laterally or sometimes eccentric. Lamella was decurrent, concolorous with the stipe and smooth edge. Shukla and Jaitly (2011) also observed that *P. djamor* produced pink colour fruiting bodies having wavy margins. The average pileus length and width of *P. djamor* measured was 4.72 cm and 7.40 cm respectively.

Prasad (2008) reported that *P. florida* had dentate margin with an average pileus length of 7.12 cm and width 7.40 cm. It had a stipe length of 4.62 cm and stipe width of 1.27 cm. Dhar *et al.* (2011) described *P. florida* as a white disc grew on a thick stipe with decurrent gills extending to the base of the stipe. It had gracious white colour, with delicate flesh and turgid texture. The pileus of *P. florida* had thin margin. The sporocarps of *P. florida* was offset and fleshy. The pileus was smooth and laterally attached to the stipe (Dung *et al.*, 2012).

P. florida produced white fruiting bodies having entire, and enrolled type pileus margins. Average pileus length and width was 6.71 cm and 7.90 cm respectively. It produced fruiting bodies with significantly longer stipe (4.80 cm) compared to *P. djamor* (0.91 cm). The average pileus thickness of *P. florida* was 6.90 mm. It had decurrent, pure white and fleshy fruiting bodies (Krishnapriya *et al.*, 2017). Whereas Jose (2018) reported the pileus size of

P. florida as 4.5-7.5 cm × 2.5-3 cm. The pileus had a spathulate shape with laterally attached stipe and its length ranged from 3 cm to 4.5 cm.

2.1.2 Microscopical Observations

Microscopical characteristics of *P. djamor* were studied by Junior *et al.* (2010). Basidiospores produced were cylindrical, thin-walled, hyaline, smooth, inamyloid and had a dimension of 8.7-11.2 µm × 3.7-5.0 µm. Basidia were 25-26 µm × 5.0-7.5 µm, clavate, four-spored, sometimes two or three spored. It produced numerous basidioles. Pleurocystidia were not observed. But cheilocystidia were present with a dimension of 18.7 – 26 µm × 6.2-11.2 µm and was subventricose to clavate. Pileus context was undifferentiated and composed of thick-walled septate hyphae (3.7-6.2 µm diameter) with clamps. Spore print obtained from *P. djamor* was pink and became white or light yellowish when dry.

Acharya *et al.* (2017) observed gill section of *P. djamor* under microscope and recorded the dimensions of basidiospores as 7.87-11.18 µm × 3.73-4.97 µm. The basidiospore was characterized by oblong, hyaline, inamyloid, thick walled cell with suprahillar depression. Basidia were narrowly clavate, tetrasterigmatic with a dimension of 20.71-21.12 µm × 4.14-4.56 µm. Pleurocystidia were absent whereas, rostrate to lecithiform cystidium like element cystidiole (25.78-29.4 × 3.73-5.73 µm) was present. Hyphal system was dimitic. Generative hyphae was hyaline to yellowish brown, thin to thick-walled whereas skeletal hyphae was blackish, and thin walled with clamp connection.

Biswas *et al.* (2011) observed that basidia of *P. florida* were 4 spored with a size of 30-38 µm × 6-10 µm. Basidiospores were oblong with 7-10 µm long. Whereas Das *et al.* (2015) measured the length and breadth of basidium to be 29.20 µm and 4.10 µm respectively. The basidiospores formed had an area of 15.68 sq. µm.

2.2 RE-ISOLATION AND PURE CULTURING OF SELECTED ISOLATES AND STUDYING MORPHOLOGICAL CHARACTERS OF THE CULTURE

Sardar *et al.* (2015) studied on the best medium for isolation of *Pleurotus* spp. and found that potato dextrose agar (PDA) was superior to all other media *viz.*, malt extract agar (MEA) and wheat extract agar (WEA). Ahmad *et al.* (2015) pure cultured the mycelium of *P. djamor* on PDA, MEA, potato yeast dextrose agar (PYDA) and yeast extract agar (YEA). Among the media used, yeast extract agar medium was found to be the best for the growth of *P. djamor*. Krishnapriya (2018) identified potato dextrose peptone agar (PDPA) medium as the best medium compared to PDA, MEA, oat meal extract agar (OMA) and carrot extract agar medium (CEA) for the three isolates, *P. cystidiosus coremial, P. cystidiosus non-coremial and P. opuntia.*

Guadarrama-Mendoza *et al.* (2014) observed a direct relationship between mycelial morphology and growth rate. Cottony mycelium presented significantly higher growth rates in comparison to floccose mycelium.

P. djamor recorded maximum growth of 75.33 mm after 8 days of incubation in 6 h light and 18 h darkness and the lowest radial growth of 30.5 mm when the light and darkness duration was same (Chauhan and Gupta, 2017).

P. florida completed its growth in 7.30 days on PDPA medium and produced pure white, thick mycelial growth (Krishnapriya *et al.*, 2017). Kumar *et al.* (2018) measured average mycelial growth of 65.36 mm on wheat extract agar six days after inoculation followed by PDA (56.01 mm) and MEA (56.01 mm). *P. florida* had maximum radial growth (63.68 mm) at temperature 22.5°C followed by 25°C, with 60.15 mm radial growth.

2.3 STUDIES ON STRAIN IMPROVEMENT BY HYBRIDISATION THROUGH SPAWN MIXING

Development of new strains could be attempted by hybridisation through spawn mixing. In such a hybridization program, the selection of appropriate parents to be used is essential (Bertan *et al.*, 2007) since, the productivity as well as quality of edible mushrooms mainly depended on the genetic makeup of the strain (Kaur and Sodhi, 2012).

Pandey *et al.* (2016) reported spawn running period of 18-19 days for *P. djamor*. It needs 6-7 days for first harvest after complete spawn run with cropping period ranging from 18-32 days. Whereas, Suresh *et al.* (2017) observed spawn running period of 12.33 days and the results were found in accordance with the findings of Satpal *et al.* (2017). In this study, it was also reported that *P. djamor* produced a maximum yield of 430.5 g kg^{-1} of dry paddy straw.

Ram and Thakur (2005) reported that in *P. florida* spawn running period ranged between 10-12 days whereas *P. djamor* had around 11 days. According to Prasad (2008), spawn running days of *P. djamor* and *P. florida* on paddy straw was 13.5 and 13 days respectively. Number of sporocarps produced as 85.38 and 75.40; average sporocarp weight as 5.54 g and 8.66 g; and BE as 47.33 and 65.35 per cent were recorded for *P. djamor* and *P. florida* respectively.

Primordial initiation in *P. florida* was reported after 16 days of mycelia colonization and harvested the same in next four days. The total cropping period ranged from 54-60 days (Ram and Thakur, 2005). The BE of *P. florida* in paddy straw substrate was 78.82 per cent (Patidar, 2008). Gaitan-Hernandez and Salmones (2008) reported, the duration for spawn ramification for *Pleurotus* species ranged from 20 to 30 days. Mondal *et al.* (2010) also observed an average of 24 days for completion of mycelial running of *P. florida* in rice straw. The pinheads appeared eight days after the complete spawn run. He also observed that the morphological characters like pileus diameter, thickness and length of

stipe decreased in successive flushes. Whereas Kalaw and Albinto (2014) recorded that *P. florida* completed spawn run and pinhead formation in 19.13 and 21.9 days respectively, with BE of 28.87 per cent on the substrate, composted rice straw and sawdust at ratio 7:3.

Manimuthu and Rajendran (2015) recorded primordial initiation of *P. florida* in 19 to 23 days and harvesting was done at 23rd, 33rd and 43rd day after bed preparation. Total cropping duration of *P. florida* was 43 days on paddy straw. According to Sumi (2016), *P. florida* had BE of 97.6 per cent on paddy straw. Patar *et al.* (2018) compared the growth performance of *P. florida* and *P. sajor-caju* grown in wheat straw substrate. The crop period reported was 43 days and 49 days respectively. *P. florida* recorded BE of 136.3 per cent whereas *P. sajor-caju* had 94 per cent.

The development of interspecific hybrids which assemble the desired characters from different strains can yield eminent strains. However, the ability to bring together such traits in one hybrid strain leads to the best aggregation of genetic material for the production of high yielding mushrooms. The recombined culture of *P. sajor-caju* and *P. florida* produced creamish sporocarp which yielded superior to one of the parent *P. florida* and on par with *P. sajor-caju* (Anitha, 1998). Whereas it was also concluded that, strain mixing by mixing of spawn of two different species does not ensure recombination as many other recombinant cultures showed poor performance compared to their parents. Chakravarty (2011) reported that mixing fertile strains produced hybrids but their identification was often difficult.

2.4 STUDIES ON STRAIN IMPROVEMENT BY HYBRIDISATION THROUGH CROSSING OF SINGLE SPORE CULTURE

A successful mushroom breeding depends on the genetic information on inheritance and major quantitative traits associated with yield, maturity period and other economic traits of breeders concern (Gregorio, 2002). The bifactorial

inheritance, observed in *Pleurotus* species, indicated the possibility of high degree of genetic variability. Cross-breeding and the development of improved strains have proved to be one among the most promising method, and thus gained a lot of popularity (Fan *et al.*, 2006; Kim *et al.,* 2011).

Bresinsky *et al.* (1987) claimed that high amount of variability was generated by intermating different strains of *Pleurotus*. Bahukhandi and Sharma (2002) observed that the monokaryon obtained in the *Pleurotus* spp. *viz. P. sajor-caju, P. sapidus* and *P. cornucopiae* could be differentiated from dikaryotic mycelium, by their slower growth, often limited to inoculum. Also these lacked clamp connection and they did not produce fruiting bodies, during cultivation. Monospores of *Pleurotus* spp. were incubated at 25°C for 7-10 days and the mycelia developed were confirmed as monokaryons, since it lacked clamp connections (Jaswal *et al.*, 2013).

Kothe (2001) confirmed two genetic loci namely A and B that control the mating type in heterokaryotic mushrooms like *Pleurotus*. Mating types of the monokaryotic isolates were analyzed using two-point inoculation technique (Kotasthane, 2003). Gharehaghaji *et al.* (2007) observed that mating compatibility between monokaryons with high radial growth was generally high, with 63 per cent or more. A lower per cent of compatibility was observed for crosses between single-spore isolates with low radial growth. Pairings were derived from compatible thick mycelium of monokaryons, whereas all crosses between fluffy monokaryotic mycelium were incompatible, thus not considered as a desirable character for mating studies. Guadarrama-Mendoza *et al.* (2014) also found that mycelial morphology could be used as criteria for the selection of compatible pairs in hybridization studies. They also suggested to select fast growing cottony single spore cultures for the production of fast growing hybrid strains.

Bahukhandi and Sharma (2002) carried out interspecific hybridization between *P. sajor-caju*, *P. sapidus* and *P. cornucopiae* for obtaining improved

strains. Based on phenotype, the resultant hybrids were categorized into two groups. First group consisted of hybrids which had similarity with their parents in appearance, yield and colour. The second group hybrids had blended characters of their parents and yield potential more or less similar to the parents. The hybrid obtained by crossing *P. sajor-caju* and *P. cornucopiae* resulted in 23.3 per cent more yield compared to their parents. The shape and size of the fruiting body was similar to *P. sajor-caju* while the colour was white resembling to *P. cornucopiae*. The hybrid completed spawn run and cropping period in 15 days and 31 days respectively. The total crop yield from the hybrid was significantly high compared to the parents and had a shorter cropping period.

Interspecies hybridization between *P. florida* and *P. eous* had been attempted by Sawashe and Sawant (2005). The hybrid cultures formed by mating showed faster mycelia growth than their parents. The average size of the hybrid fruiting bodies was intermediate of both the parents, while the stipe length and average weight increased with respect to *P. eous* but was on par with *P. florida.*

Prasad (2008) studied the morphological and biochemical variability of five *Pleurotus* spp. *viz. P. sajor-caju*, *P. djamor* var. *roseus, P. florida, P. flabellatus* and *Hypsizygus ulmarius*. On the basis of yield and quality parameters, four *Pleurotus* hybrids *viz.* A1B5 (*P. sajor-caju* x *P. djamor*), A1C2 (*P. sajor-caju* x *P. florida*), A11D6 (*P. sajor-caju* x *P. flabellatus*) and F4H4 (*P. florida* x *H. ulmarius*) were selected. Hybrid A1B5 produced fruiting bodies with long stipes (4.50 cm), while hybrid A1C2 produced fruiting bodies with maximum pileus width (8.20 cm) which was significantly longer than the parents. All the hybrids yielded significantly superior compared to their respective high yielding parents. Among the hybrids, A1C2 found to be the best which produced maximum yield (1536.90 g kg^{-1} dry substrate) and number of sporocarps (140.6) with higher protein content (28.75 %) and lower total soluble sugars content (15.25 %).

Kumara and Edirimanna (2009) observed that the hybrids obtained from American and Lanka oyster had no significant difference in the morphology of fruiting body and the BE was found to be low. Jaswal *et al.* (2013) conducted interspecific hybridisation between *P. florida* PAU-5 and *P. sajor-caju* PAU-3. Out of 35 *Pleurotus* hybrids, 20 resembled with the parent *P. florida* PAU-5 while 8 were close to *P. sajor-caju* PAU-3 with slight difference in pileus morphology. Five *Pleurotus* hybrids were identified based on yield and desirable characters. *Pleurotus* hybrid no. 8 and 37 exhibited morphology different from the parent strains while *Pleurotus* hybrid no. 16 and 46 were similar to the parent *P. sajor-caju* PAU-3 but had higher whiteness index. *Pleurotus* hybrid no. 42 was morphologically similar to *P. florida* PAU-5 and had 51 per cent higher BE.

Guadarrama-Mendoza *et al.* (2014) studied on two native *Pleurotus* spp. strains, white LB-050 and pale pink LB-05. These were chemically dedikaryotized to obtain their symmetrical monokaryotic components. The taxonomic study revealed that LB-050 belongs to *P. djamor* var. *djamor* while LB-051 belongs to *P. djamor* var. *roseus*. As a result of hybridization, 44.6 per cent of the hybrid strains were white resembling LB-050 strain and 26.80 per cent of the hybrid strains inherited the pink pale colour of LB-051 strain. The remaining 28.60 per cent of hybrids had an off-white colour. The result suggested that pairing of white monosporous cultures always produced white mycelia, whereas pale pink monosporous culture paired with white ones produced pale pink mycelia.

Tagavi *et al.* (2016) hybridised *P. florida* and *P. eryngii*, and found that only four combinations were consistent and successful, which were named as H1, H32, H11 and H40 hybrids. H40 was the best hybrid based on cap diameter, dry and fresh weight of fruiting body, yield and biomass; and was morphologically similar to *P. eryngii*.

Abdulgani *et al.* (2017) carried out hybridisation between *P. pulmonarius* and *P. citrinopileatus*. The hybrids developed had superior qualities of

P. pulmonarius viz., large fleshy pileus with rigid stipe and cluster-type growing pattern of *P. citrinopileatus*. Besides, the hybrids had shorter spawn run period, higher yield and increased BE compared to the two parental strains. Phenotypically the hybrid strains were more similar to *P. pulmonarius*. The hybrid P19 x C5 had the superior characteristics like faster mycelial growth and thick colony; and produced sporocarps with fleshy texture, bigger pileus and good aroma. Spore print analysis of the hybrid P19 x C5 revealed that the spore load was low compared to *P. pulmonarius*.

2.5 STUDIES ON STRAIN IMPROVEMENT BY GAMMA IRRADIATION

Mushrooms were subjected to mutation studies to obtain better quality traits and productivity. Through induced mutation by ionizing radiation, desirable strains were selected from diverse strains (Fan *et al.*, 2006). The desired mutant strains were economically beneficial *viz.,* resistance to pathogen and pests, higher yield, etc. (Djajanegara and Harsoyo, 2008).

Strong ionizing radiation like X-rays and gamma rays cause single- and double-stranded breaks in the DNA backbone through the formation of hydroxyl radicals on radiation exposure. Ionizing radiation modified bases; for example, the deamination of cytosine to uracil (Tindall *et al.*, 1988).

Anitha (1998) reported that gamma radiation in *P. florida* culture at 2 and 2.5 kr level resulted in higher yield than other levels of irradiation ranging from 0.25 kr to 2.5 kr. Compared to basidiospores of *Pleurotus* spp., genetic similarities of the mycelium were altered easily and dose dependently by gamma irradiation (Young-Keun and Hwa-Hyoung, 1999). To induce the lignocellulolytic mutants of *P. ostreatus*, the mycelia was gamma irradiated at 1 and 2 kGy. The isolated strains exhibited 10 times higher extracellular enzyme activities in liquid media compared to the control (Lee *et al.*, 2000).

Grodzinskaya and Mikheev (2001) observed three to five days of acceleration in primordial formation at minimum (25 Gy) and maximum (200 Gy)

dose of irradiation in *P. ostreatus*. It was concluded that two levels of stimulating doses might be due to two mechanisms of radio-stimulation related to reparation processes or acceleration of vegetative growth.

Ravichandran and Muthusamy (2005) exposed spores of *P. florida* at 3 kr to get maximum yield, and above 3 kr, there was reduction in yield. In *P. florida*, three putative mutants were developed by Djajnegara and Harsoyo (2008) using gamma rays at 0.75 kGy with dose velocity of 1.149 kGy h^{-1}. Among three, one mutant had significantly higher antioxidant content and the other had higher productivity compared to the control. In order to induce mutants with improved characteristics, Beejan and Nowbuth (2009) selected five parent strains of *Pleurotus*. The selected strains were two *P. sajor-caju* strains, CC 46 and CC 116, which had good performance at low temperatures (16°-19°C), *P. columbinus* (CC 66) and *Pleurotus* hybrid (CC 71) having good mycelial growth rate at higher temperatures (28°-31°C) and the commercially cultivated *P. sajor-caju* CC 114. These were subjected to gamma irradiation ranging from 5 to 400 Gy using a ^{134}Cs radioisotope. Stimulatory effects on mycelial growth rate and crop yield were observed at the irradiation dose 0.2 kGy. Gamma irradiation did not result in any change in pileus colour and morphological characters of the selected isolates. However, the observed beneficial effects were not consistent.

Irradiation of *P. ostreatus* by 1 kGy resulted in an increased metabolism, glucan synthesis, BE and energy content (Dawoud and Taleb, 2011). Rashid *et al.* (2014) inferred that the recommended dose of gamma irradiation for mutation induction in mycelium of *P. sajor-caju* was less than 2.2 kGy. When irradiated at 0.6 kGy, the promising mutants increased BE over the parent. The results also revealed that, growth rate of irradiated mycelium were slightly lower than the control but the growth rate decreased significantly as the dose increased. Whereas BE of *P. sajor-caju* increased by 23.10, 33.68, 38.05, 36.80 and 39.82 per cent as the irradiation dose increased from 0 (control), 0.1, 0.2, 0.4 and 0.6 kGy (Rashid *et al.*, 2016). Irradiation also resulted in increased number of fruiting bodies, with no significant difference in size of fruiting bodies.

Mycelia of straw mushroom were gamma irradiated at 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 kGy (Sermkiattipong and Charoen, 2014). Among 13 isolates of stable mutants, No.0.25-17 irradiated at 0.25 kGy was the best mutant with significantly high BE with a shorter cultivation period over the parent strain.

Mycelial growth of milky mushroom cultures treated with low dose (10 Gy) was faster than that of unirradiated cultures while high doses (250, 500,750, 1000 Gy) inhibited the mycelial growth significantly. One among the mutant CI-2, which was irradiated at 10 Gy completed the mycelial growth in a shorter time (9.00 days). As radiation dose increased over 250 Gy, the growth rate gradually decreased and reached minimum at 1000 Gy (Abou-Elftouh *et al.*, 2016).

2.6 COMPARISON OF THE BEST CULTURES

2.6.1 Yield

Dhar (2018) conducted interspecific hybridisation in *Pleurotus* spp. and developed hybrids of *P. sajor caju* x *P. flabellatus*, *P. djamor* x *P. sajor caju* and *P. djamor* **x** *P. flabellatus*. All the third generation selected hybrids between the three combinations were compared to their parents. Hybrids produced pinheads in minimum days with improved yield compared to their parents.

2.6.2 Proximate Constituents of *Pleurotus* spp.

Oyster mushrooms have been cultivated since ancient times for their nutritional value, flavour and texture. Chattopadhyay *et al.* (2014) claimed that *Pleurotus* spp. was rich in carbohydrates, proteins, vitamins, minerals and crude fibres.

2.6.2.1 Estimation of Moisture Content

Generally mushrooms contain 90 per cent water and 10 per cent dry matter. The moisture percentage of *Pleurotus* spp. varied among different species and it depended on parameters like stage of harvest, growing environment and

storage conditions (Reis *et al.*, 2012). Agarwal *et al.* (2017) reported the moisture content in oyster mushrooms ranged from 85 to 90 per cent.

P. florida cultivated in cotton waste material recorded 77 per cent moisture content (Khan *et al.*, 2011). Ashraf *et al.* (2013) reported that *P. djamor* had a moisture per cent of 82.77, when cultivated in paddy straw. Bora and Kawatra (2014) recorded 91.80 per cent moisture content in *P. florida*. Similar result in *P. florida* (91.50 %) was reported by Maftoun *et al.* (2015) and for *P. djamor* moisture content recorded was 82.21 per cent. On fresh weight basis, the moisture content of *P. citrinopileatus, P. florida* and *P. sajor-caju* was 90, 90.8 and 87.84 per cent respectively (Shevale and Deshmukh, 2016). Jose (2018) recorded the moisture per cent of 66.95, 84.92, 88.38, 89.28 and 91.10 in *Ganoderma lucidum*, *P. florida, P. djamor, H. ulmarius and Calocybe indica* respectively.

2.6.2.2 Estimation of Carbohydrate

Carbohydrate constituted the major component (46.6-81.81 %) in dry matter of *Pleurotus* spp. (Bano and Rajarathnam, 1982). It is present mainly in the form of polysaccharides, chitin, glucans and hemicellulose. Khan and Tania (2012) reported that polysaccharide content of *Pleurotus* spp. ranged from 36 to 60 g per 100 g of dry weight. Similarly, Deepalakshmi and Mirunalini (2014) recorded 50-60 per cent carbohydrate content in *Pleurotus* spp.

Alam *et al.* (2008) estimated the carbohydrate content of *P. ostreatus* (37.80%), *P. sajor-caju* (39.82%), *P. florida* (42.83%) and *C. indica* (48.50%). Similarly, Ahmed *et al.* (2009) also reported 55.50 per cent carbohydrate content on dry weight basis in *P. florida*. Whereas, Pushpa and Purushothama (2010) and Menaga *et al.* (2012) recorded comparatively lower carbohydrate content *i.e.,* 32.08 per cent and 26.6 per cent in *P. florida* on dry weight basis. Carbohydrate content of *P. djamor*, pink *Pleurotus* isolate, white *Pleurotus* isolate and *P. cystidiosus* were 50.59, 51.41, 53.22 and 33.43 per cent respectively (Pandey

and Veena, 2012). Ashraf *et al.* (2013) reported 37.69 per cent carbohydrate content on dry weight basis in *P. djamor*. Likewise, Bora and Kawatra (2014) recorded 47.20 g per 100 g of *P. florida*. Selvakumar *et al.* (2015) analysed the carbohydrate content in *P. ostreatus* var. *florida*, *P. djamor* var. *roseus* and their hybrid as 49.80, 45.20 and 50.10 per cent respectively. On different sawdust, *P. ostreatus* had carbohydrate content ranged from 39.67 to 42.36 per cent (Bhattacharya *et al.*, 2015). Maftoun *et al.* (2015) recorded 58 and 59.90 per cent carbohydrate in *P. florida* and *P. djamor* respectively. Jose (2018) reported that *G. lucidum*, *P. florida, P. djamor, H. ulmarius and C. indica* had 39.19, 26.68, 26.59, 30.87 and 46.17 per cent carbohydrate content respectively.

2.6.2.3 Estimation of Protein

Edible mushrooms are considered as 'poor man's protein'. Protein content in *Pleurotus* spp. ranged from 8.9 to 38.7 per cent on dry weight basis (Kurtzman, 2005). Chang (2008) reported that mushrooms ranked above vegetables, fruits and milk in protein content.

Alam *et al.* (2008) reported 23.91, 24.63, 20.56 and 21.40 per cent protein content on dry weight basis in *P. ostreatus, P. sajor-caju, P. florida and C. indica* respectively. Pandey and Veena (2012) recorded that *P. djamor,* pink *Pleurotus* spp., *P. cystidiosus* and white *Pleurotus* spp. contained 29.81, 30.87, 24.56, 24.68 and 36.25 per cent protein content respectively. Khatun *et al.* (2015) reported that *P. florida* had higher protein content (23.80%) followed by *P. citrinopileatus* (20.80%) and *P. pulmonarius* (16.80%). Protein content of *H. ulmarius*, *P. florida, P. sapidus, P. citrinopileatus, P. djamor, P. flabellatus* and *P. platypus* were 33.60, 33.5, 32.50, 32.30, 30.60, 30.30 and 28.30 per cent respectively (Mishra *et al.*, 2015).

Ahmed *et al.* (2009) reported that *P. florida* cultivated in paddy straw had 22.40 per cent protein content on dry weight basis. Similar reports on protein content (23.18 %) was reported by Pushpa and Purushothama (2010). Khan *et al.*

(2011) recorded that protein content in dried fruiting bodies of *P. florida* was 38.86 per cent, when cultivated in cotton waste material. Whereas, higher protein content of 50.70 per cent in *P. florida* was reported when cultivated in paddy straw (Menaga *et al.*, 2012). According to Bora and Kawatra (2014) crude protein content of *P. florida* was 27.29 per cent on dry weight basis.

Selvakumar *et al.* (2015) estimated the proximate composition of protein in *P. ostreatus* var. *florida*, *P. djamor* var. *roseus* and their hybrid, cultivated in paddy straw. Maximum amount of protein content was recorded in the hybrid (29.40%) followed by its parents, *P. ostreatus* var. *florida* (27.90%) and *P. djamor* var. *roseus* (25.10%).

Shukla and Jaitly (2011) reported 20.83 and 20.40 per cent protein content in *P. florida* and *P. djamor*. According to Ashraf *et al.* (2013), protein content estimated in *P. djamor* was 24.83 per cent. Maftoun *et al.* (2015) recorded higher protein content in *P. florida* (27 %) compared to *P. djamor* (15.60%).

2.6.2.4 Estimation of Crude Fibre

Alam *et al.* (2008) claimed that *Pleurotus* spp. were good source of dietary fibre, due to the presence of non-starch polysaccharides.

Khan *et al.* (2008) reported 26.20, 27.00, 26.80, 25.50 and 26.30 per cent fibre content on dry weight basis in *P. sajor-caju, P. ostreatus, P. florida, P. cystidiosus* and *P. geestaranus* respectively. Alam *et al.* (2008) recorded the fibre content in *P. ostreatus* (24.34%), *P. sajor-caju* (22.87%), *P. florida* (23.29%) and *C. indica* (12.9%). Crude fibre content of *P. djamor,* pink *Pleurotus* sp., white *Pleurotus* sp. and *P. cystidiosus* estimated by Pandey and Veena (2012) was 13.20, 27.80, 5.10 and 4.90 per cent respectively.

Crude fibre content of *P. florida* was estimated as 8.10 per cent (Ahmed *et al.*, 2009). Whereas, Pushpa and Purushothama (2010) reported higher

crude fibre content (23.18%) in *P. florida*. According to Bora and kawatra (2014), the crude fibre content in *P. florida* was 11.87 g per 100g of dry weight.

Ashraf *et al.* (2013) reported that *P. djamor* contained 22.03 per cent crude fibre. Whereas, Maftoun *et al.* (2015) estimated the crude fibre content in *P. florida and P. djamor* as 11.50 and 17.20 per cent respectively. Usha and suguna (2015) reported that fibre content in blue oyster mushroom ranged from 17.45 to 19.45 per cent. The fibre content of *P. ostreatus* var. *florida, P. djamor* var. *roseus* and their hybrid was analysed and recorded as 7.56, 9.10, 7.85 per cent respectively (Selvakumar *et al.,* 2015).

2.7 INCIDENCE OF PESTS AND DISEASES

Fungal and bacterial contamination was one among the major constraints in spawn and mushroom production faced by mushroom growers all over the world. Such infections were facilitated by the conditions under which mushroom cultivation was commonly carried out such as warm temperatures, humidity, carbon dioxide (CO_2) and presence of pests (Bellettini *et al.*, 2018).

Jaivel and Marimuthu (2010) reported that Trichoderma, Aspergillus and Rhizopus were the predominant microorganisms on oyster mushroom bed and their occurrence was severe in summer and spring seasons, than autumn and winter. While, Biswas and Kuiry (2013) observed that *Aspergillus niger*, *Coprinus* sp., *Penicillium* sp., and *Sclerotium rolfsii* were the most predominant fungal contaminants of mushroom beds of *P. florida*.

Biswas (2016) also reported the occurrence of contaminants *viz. T. harzianum, Penicillium notatum, A. niger, Coprinus* spp., *Mucor* sp., *Rhizopus* sp., and *S. rolfsii* in mushroom beds. It was also found that, *T. harzianum, P. notatum, S. rolfsii* and *Coprinus* spp. were the most dominant fungal contaminants. The incidence of the contaminants were minimum during January (2.86%) and it was increased considerably with the fluctuating climatic conditions and reached its peak during June (32.8%). Thereafter, a decline trends in

contamination per cent was noticed. In conformation with the above observations, Kumar and Sarathi (2017) also noted the occurrence of eight contaminants in *Pleurotus* mushroom beds and out of which *T. viride*, *A. niger*, and *Coprinus* sp. were found to be the dominant fungal contaminants. The incidence was high during May to July (23.5 - 26.7%) and it caused maximum loss to mushroom yield. The incidence of contaminants were minimum during December and January (3.60%) and maximum during the month of May (26.5 %). Qiu *et al.* (2017) isolated *T. asperellum* from contaminated substrate of *P. ostreatus* with green mold disease. They found a positive correlation between high temperature and colonization of mushroom mycelia by *T. asperellum*. Krishnapriya (2018) observed fungal contaminants like *Trichoderma* sp., *Aspergillus* sp. and *Penicillium* sp. during cultivation of *P. cystidiosus* and *P. opuntiae*.

Lim *et al.* (2008) isolated eight distinct bacterial genera *viz.*, *Bacillus, Enterobacter, Sphingomonas, Staphylococcus* and *Moraxella* from the diseased mycelia of *P. eryngii*. According to Kim *et al.* (2015), the bacterial pathogen *Pantoea* sp. caused severe soft rot disease in king oyster mushroom, *P. eryngii*. The symptoms included water-soaked lesions and soft rot.

Another limiting factor for the proper development of mushrooms were the presence of insects, mites, crustaceans, mycetophagous arthropods and wood substrate decomposers. Singh and Sharma (2016) documented major insect pests of *Pleurotus* mushrooms as phorids, cecids, mites (tarsonemid-mites, red pepper and tyroglyphid mites), springtails and beetles. Jose (2018) observed insects like *Megaselia* sp., *Lycoriella* sp. and thrips on the beds of oyster and milky mushroom. Sumi (2016) recorded insect pests like, phorid flies, staphylinid beetles and springtails during the cultivation of *H. ulmarius*. Occurrence of pests like phorid flies, springtails, black ants and staphylinid beetles were also reported by Krishnapriya (2018) on *P. cystidiosus* and *P. opuntiae*.

Flies which belonged to eight dipterous families Calliphoridae, Culicidae, Drosophiladae, Cecidomyiidae, Muscidae, Mycetophilidae, Phoridae and Sciaridae infested mushroom and transmitted diseases in mushroom. They derived nutrients from fruiting bodies and carried fungal contamination, bacteria diseases and mites. Another most dangerous pests were nematodes. Button mushrooms were susceptible to nematode infestation while oyster mushrooms were relatively resistant. *Tarsonemus* spp. and *Histiostoma* spp. were the major mushroom damaging mites. Their population build up was maximum under high humidity of 90 per cent and at temperature 25-30 °C. Webs were formed between the cultivation shelves and fruiting body. Mites fed on mycelia and fruiting bodies, causing reduced yield and quality of mushroom (Bellettini *et al.*, 2018).

2.8 RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

The variations in new strains, owing to considerable developmental plasticity of fungi, are not always expressed in terms of morphological divergence. The morphological characters of a fruiting body were influenced by environmental conditions (Rabbani *et al.*, 2010). Also some isolates of *P. ostreatus* complex belonging to different species showed morphological similarity (Asef, 2012). Hence the classification entirely based on morphological characteristics is not reliable.

The limitation of identification of mushroom strains based on a few morphological features can be overcome by use of modern molecular techniques. Nowadays different techniques like molecular markers of rDNA sequencing, RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), microsatellite and mitochondrial genotypes are used to discriminate mushroom strains. These technologies provide reliable data for mushroom strain identification and protection. Avin *et al.* (2012) stated that such molecular markers were used to verify the taxonomic position of the strains. Thus molecular biology techniques provided a useful and a reliable methodology for

systematic classification and analysis of genetic polymorphism in mushrooms (Avin *et al.*, 2016). RAPD fingerprinting had been used for genetic diversity analysis, varietal identification and strain protection in various field crops (Yadav *et al.*, 2017).

For discrimination of various mushroom strains, Rehman *et al.* (2015) used RAPD markers. They recommended that the marker assisted in strain identification and also in protection of elite strains. According to Yadav *et al.* (2017) RAPD analysis was the most sensitive and powerful tool for genetic variation assessment at DNA level among *Pleurotus* species.

RAPD analysis was first developed to detect polymorphism between organisms, to produce genetic markers and to construct genetic maps (Williams *et al.*, 1990). High level of genetic diversity got revealed when RAPD markers were used for genetic similarity analysis and grouping. Khush *et al.* (1992) used RAPD analysis to differentiate the strains of a variety of homobasidiomycete mushrooms.

A study on radiation sensitivity using RAPD revealed that 2 kGy of gamma irradiation in the basidiospores of *P. ostreatus* changed 22-25 per cent of genetic similarity whereas 23-36 per cent in the irradiated mycelia (Young-Keun and Kwa-Hyoung, 1999).

The RAPD patterns of the control and five strains (PO-5, PO-6, PO14, PO-15 and PO-16) of *P. ostreatus* gamma irradiated at 1 and 2 kGy were analysed by Lee *et al.* (2000) using ten 10-base primers. Ten primers produced polymorphism in all six strains tested. It produced 3-8 bands ranging from 0.3 to 4.5 kb. The genetic similarities of the three strains PO-5, PO-6 and PO-14 decreased to 93, 91 and 73 per cent of the control, respectively. The genetic similarities of the two strains PO-15 and PO-16 from 1 kGy re-irradiation group of PO-14, decreased to 91 and 82 per cent of PO-14 strain and to 77 and 64 per cent of the control, respectively.

Staniaszek *et al.* (2002) analyzed the genetic relationship between 26 strains of *A. bisporus* using RAPD method and reported that there was great genetic similarity among the examined strains.

Chandra *et al.* (2010) determined the genetic divergence of eight Indian species of *Pleurotus* based on RAPD pattern. Result revealed that all the species tested were differentiated by RAPD analysis and even one individual primer (OPD-07) alone discriminated all the tested species. Genetic similarity analysis and grouping also revealed a high level of genetic diversity. Sarker *et al.* (2011) analysed RAPD fingerprinting of four different species of oyster mushroom *i.e., P. djamor, P. florida, P. ostreatus* and *P. salmoneostramineus*. Based on the dendrogram drawn, two major clusters C1 and C2 were generated. Out of six random primers, the maximum polymorphism was observed by the primer OPA 09 (18.18%). The two species, *P. djamor* and *P. ostreatus* were observed to be least similar having value 43.00 per cent.

Gupta *et al.* (2011) observed very large diversity at both phenotypic and genotypic level when 42 isolates (10 di- and 32 monokaryotic isolates) of *Pleurotus* spp., *P. sajor-caju, P. florida, P. eous* and one wild relative of *Pleurotus* called *H. ulmarius* were analysed with RAPD genetic markers. It provided the basis for monokaryon selection for the development of high yielding hybrids. Khan *et al.* (2011) analysed seven strains of Oyster mushrooms, *P. platypus* (P-6), *P. flabellatus* (P-7), *P. florida* (P-17), *P. ostreatus* (P-19), *P. sajor-caju* (P-56), *P. warm-stram* (P-9) and *P. eryngii* (P-16) using RAPD. Out of 14 random primers, the maximum polymorphism was observed by primers OPL3 (72.70 %) and OPL11 (70%). The dendrogram based on RAPD analysis generated three clusters A, B and C. The two species P-56 and P-17 were observed to be the most similar having 86 per cent similarity.

Mishra *et al.* (2012) in his study on ten *Pleurotus* species, *P. sajor-caju, P. flabellatus, P. platypus, P. fossulatus, P. florida, P. citrinopileatus, P. sapidus, P. djamor, P. ostreatus* and *H. ulmarious*, observed unique banding pattern

obtained from 40 decamer primers. He also observed that, some of the RAPD primers were specific in distinguishing the *Pleurotus* species. RAPD markers showed polymorphism percentage of 98.69 and genetic similarity ranging from 22 to 98 per cent. Genomic discrimination of nine commercial mushrooms *A. bisporus, P. eryngii, L. edodes, H. tessellatus, F. velutipes, P. ostreatus, P. djamor, C. indica* and *P. florida*, by DNA fingerprinting using RAPD marker was studied by Agarwal *et al* (2013). Cluster analysis was performed with weighted pair-group with arithmetic average (UPGMA) by Alpha Imager HP. Two main clusters were observed in the dendrogram, where *P. ostreatus* exhibited least similarity with all the other samples, whereas *P. florida* and *P. djamor* shared same morphological or physiological characteristics.

A new hybrid strain of *P. eringii* along with its parents were amplified with RAPD primers and resulted in three polymorphic amplicons in the hybrid. This ensured the hybridity as well as the identification of new hybrid strain (Kim *et al.,* 2013). Abdulgani *et al.* (2017) carried out hybridisation between *P. pulmonarius* and *P. citrinopileatus*. Further analysis via RAPD confirmed that the five hybrids developed had high genetic homology with *P. pulmonarius*.

Yin *et al.* (2013) observed 94-99 per cent polymorphism in genetic diversity of 15 Chinese *P. pulmonarius* cultivars. The genetic variability, pileus colour and yield of four commercial strains of *P. ostreatus* POS 98-38, POS 09-100, POS 09-101 and POS 09-102 were evaluated using RAPD markers (Vieira *et al.*, 2013). Molecular characterization exhibited two different groups, with 69 per cent similarity between them. Within the group containing three sample strains POS 09-100, POS 09-101 and POS 09-102, it had similarities of 93 per cent, which recorded light grey colour pileus and yield statistically different, relative to the second group. The second group was formed only by strain POS 98-38, which had dark grey pileus and lower yield than the first group. A total eight *Pleurotus* spp. when tested for their genetic variability by RAPD analysis, using six random primers, diversities ranged from 30 to 70 per cent (Yadav *et al.*, 2017). When Khan *et al.* (2017) analysed seven strains of Oyster

mushrooms, similarity index ranged from 45 to 72 per cent whereas polymorphism ranged from 0 to 83.33 per cent, with an overall polymorphism of 58 per cent.

Materials and Methods

3. MATERIALS AND METHODS

The experimental studies pertaining to "Development of improved strain in Oyster mushroom (*Pleurotus* spp.)" was carried out at Department of Plant Pathology, College of Agriculture, Vellayani, during the period 2017-2019. The objective of the study was to develop an improved strain from native isolates of species of *Pleurotus*. The methodologies used for the studies are described below.

3.1 MORPHOLOGICAL CHARACTERISATION OF SPOROCARP OF NATIVE ISOLATES

The cultures of native isolates, namely *P. djamor*, *P. florida* and an isolate having 80 per cent similarity with *P. florida* were collected from mushroom unit of Instructional farm, College of Agriculture, Vellayani. These cultures were used for spawn production and bed preparation, to study the morphological characteristics of sporocarps of *Pleurotus* spp.

The spawn of the native isolates were made as per the standard procedure (Sinden, 1934). Paddy grains were used as substrate for spawn production. Unbroken grains free from diseases and insect damage were thoroughly washed in clean water three to four times to remove soil debris, straw particles, dust, chaff etc. Washed grains were then soaked in sufficient water for 20-30 minutes and were cooked in boiling water until the seed coat just begun to split open. Full opening of the grain was avoided, as it favoured the growth of microbial contaminants. The grains were spread on a clear area for drying, after draining the excess water. The boiled grains after sufficient drying were mixed with calcium carbonate at the rate of 40 g kg^{-1} of grains, to maintain the pH of the grains around 7 to 7.8 and to avoid sticking together. These were packed in polypropylene bags (12"×6") at the rate of 300 g per bag and sterilized by autoclaving at 1.02 kg cm^{-2} pressure and 121^{0}C for 2 h. After cooling, the bags were inoculated aseptically with mycelial bits of size 1cm x 1 cm from 10 days

old cultures of species of *Pleurotus* and incubated at room temperature (26 $\pm$ 2 ^{0}C) until the mycelium completely covered the grains.

Mushroom beds were prepared as per the procedure described by Bhaskaran *et al.* (1978). Paddy straw was sterilized chemically by soaking in 100 L of water containing 7.5 g carbendazim and 50 mL formalin for 18 hours. Then the excess water was drained off and the straw was spread over a clean silpaulin sheet under sun to reduce the moisture content to 60 per cent. The beds were prepared in polythene bags of 60 cm x 30 cm size. Paddy straw was twisted and placed in bag. Spawn was laid over the twist towards the sides, over which paddy straw twists were again laid and spawning was done. Likewise, four layers were prepared and the upper layer was fully spread with spawn. Each bag was filled with 1 kg straw (dry weight) and 150 g of spawn. The bags were made compact, tied at the top and provided with around 15 pin holes for air circulation. The beds were incubated in dark room with adequate aeration for the spawn run. After complete spawn run, slits were cut on the bed for primordial initiation. The fruiting bodies produced were then used for recording the macroscopical and microscopical observations.

3.1.1 Macroscopical Observations

The sporocarps were observed for the macroscopical characters like pileus colour, length, diameter, shape, texture, stipe length, stipe width, number of gills per centimetre, gill colour and its attachment. The characters observed were recorded based on the data sheet provided by Nair (1990) as given in Appendix I.

The various developmental stages of species of *Pleurotus* were also recorded. The change in colour, size and shape of the isolates from pinhead to maturity stage was observed.

3.1.2 Microscopical Observations

Microscopical observations were recorded as per the standard technique (Deepa, 2016), using fresh matured sporocarp of species of *Pleurotus*. Thin sections of the gills were cut and stained with 1 % congo red stain (Appendix II). The dimensions of basidia and cystidia were measured using compound microscope (Carl Zeiss Primo star, Germany) at 1000X magnification. To determine the shape of basidia and cystidia, microphotographs were also taken.

For microscopic studies of mycelium of species of *Pleurotus*, standard technique of slide culture (Riddell, 1950) was followed. Glass slides, coverslips and glass rod were placed inside petri plates with blotter paper. The unit was sterilized in autoclave at 121°C and 1.05 kg cm^{-1} pressure for 15 min. Then the unit was taken inside laminar air flow chamber. The blotter paper was moistened with sterile water and two glass rods were placed above the blotter paper. Above the rods, glass slide was kept horizontally. A piece of 10 mm x 10 mm size plain agar was placed at the centre of glass slide and then four sides were inoculated with the cultures of species of *Pleurotus*. A coverslip was placed over the agar piece and incubated at room temperature 26 $\pm$ 2^{0}C, until the growth has occurred. Then the coverslip was gently lowered onto the small drop of lactophenol cotton blue (Appendix II) placed on a clean microscope slide.

Spore prints of the three isolates were made. A half matured sporocarp of species of *Pleurotus* was selected for obtaining the spore print. The pileus was carefully detached from the stipe without bruising the gills and sporocarp. It was laid flat with the gill side facing towards the surface of a black chart paper for 24 hour. To avoid air current and also to provide a humid environment for the easy discharge of the spores, a bell jar was placed over the sporocarp. After 24 h, the bell jar was removed and the pileus was taken off to obtain the spore print. The colour of the spore dust was noted and the spore prints were preserved. To study the microscopic characteristics, basidiospores obtained from the spore print were stained with 1 % congo red and were examined under compound microscope.

The dimensions of spores were measured using compound microscope at 1000X magnification. Microphotographs of spores were also taken to examine the shape.

3.2 RE-ISOLATION AND PURE CULTURING OF SELECTED ISOLATES AND STUDYING MORPHOLOGICAL CHARACTERS OF THE CULTURE

Re-isolation of the selected isolates *viz., P. djamor*, *P. florida* and the isolate with 80 per cent similarity with *P. florida*, was made using the standard tissue culture technique (Suharban, 1987). Pest and disease free, and partially matured, healthy mushroom sporocarps were selected for tissue culturing. Sporocarp was thoroughly cleaned and surface sterilized with ethyl alcohol (99.9 %). Sporocarp was then split longitudinally and a small tissue from the junction of pileus and stipe was detached using a sterile inoculation needle. Tissue was then aseptically transferred to petri plates containing PDPA medium and incubated for 7-10 days at room temperature (26 $\pm$ 2^0C). The chemical composition of PDPA is given in Appendix III.

Cultures obtained from tissue culturing were further purified using hyphal tip culture method (Rangaswamy and Mahadevan, 2008). The hyphal tips of developing mycelial cultures were aseptically transferred to test tube slants of PDPA medium. Pure cultures were incubated at 26 $\pm$ 2^0C for 15 days. The growth was revived subsequently at periodical intervals of 25-30 days. For long term preservation, the cultures were stored in refrigerator at 4°C and revived at trimonthly interval.

3.2.1 Cultural Characterization

The pure cultures were subcultured in petri plates (9 cm) containing solidified sterile PDPA medium (20 mL). Five mm discs were cut from the periphery of actively growing culture with the help of a sterile cork borer and were aseptically placed at the centre of petri plate containing the media. The cultures were incubated under room temperature (26 $\pm$ 2^0C) and were observed for

colour, growth pattern and density. Mycelial growth rate was determined by measuring the average of mean daily growth until the culture was grown fully in the petri plate. The average reading was plotted against time (day) to obtain the growth rate in terms of mm day^{-1} (Guadarrama-Mendoza *et al.*, 2014). Four replications were maintained for each isolate of species of *Pleurotus*.

3.3 STUDIES ON STRAIN IMPROVEMENT BY HYBRIDISATION THROUGH SPAWN MIXING

The experiment was laid out in completely randomized design (CRD) with five treatments. Four replications were maintained for every treatment. Each replication denoted five mushroom beds. The crop was laid during November 2018 and the weather data is given in Appendix IV.

Mother spawn of the three isolates *viz., P. djamor*, *P. florida* and the isolate with 80 % similarity with *P. florida*, were prepared and incubated at 26 ± 2°C. After completing full spawn growth (on 20th day), spawn mix was done by mixing spawn of different species in equal proportion (75 g each). Isolate 1 was mixed thoroughly with isolate 2 and isolate 3 separately. Using the mixed spawn, mushroom beds were laid as 3.1 on paddy straw (Figure 1). Along with this, control beds were also laid with the three isolates.

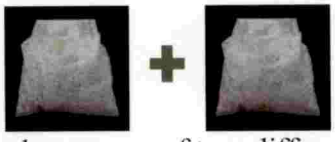
The mushroom beds were maintained in cropping room under optimized range of temperature and humidity at 27-30^{0}C and 80-90 per cent respectively with fresh air circulation (Islam *et al.*, 2017). The following observations were recorded for comparative performance.

1. Time taken for complete spawn run as the days required for the complete mycelial growth on the mushroom bed
2. Time taken for pinhead formation as the days required for the initiation of pinhead on mushroom beds
3. Days taken for first harvest as the days taken by the pinhead to attain maturity and ready for harvest

Selection of mushroom sporocarp with desired character

Production of mushroom culture by tissue culturing of sporocarp

Mother spawn production from the mushroom culture



Mixing equal quantity of mother spawn of two different *Pleurotus* spp.

Preparation of mushroom bed using mixed spawn

Selection of sporocarps with desirable characters and production of mushroom culture by tissue culturing

Repeat the cultivation for three generation and select the stable recombinant with desired characters

Figure 1. Steps for preparation of mixed spawn of species of *Pleurotus*

4. Total yield from three harvests as total weight of the mushrooms harvested from the bed
5. Total crop period as the total number of days taken from the spawn inoculation till the last harvest of mushroom
6. Average weight of sporocarp as the weight of sporocarps from first two harvest divided by the number of carp obtained
7. Number of sporocarp as the total number of sporocarp harvested upto three harvest
8. Biological Efficiency (BE) (per cent) is calculated using the formula,

$$\text{BE (per cent)} = \frac{\text{Total fresh weight of mushroom harvested per bag (g)}}{\text{Dry weight of substrate per polybag (g)}} \times 100$$

9. Incidence of pest and diseases during the time of spawn run as well as mushroom production

3.4 STUDIES ON STRAIN IMPROVEMENT BY HYBRIDISATION THROUGH CROSSING OF SINGLE SPORE CULTURE

3.4.1 Preparation of Monospore Culture

The serial dilution method demonstrated by Bahukandi and Sharma (2002) was followed to get the monosporous cultures of the three species. Half matured healthy sporocarp was selected for the single spore isolation. The stipe of the sporocarp was cut using a sterile blade. Pileus of each strain was then separately kept in sterilized petri plate such that the gill surface faces the downward position of the sterile petri plate. After half an hour, pileus was removed and the spores were collected. The spore suspension was prepared by adding the collected spores in 10 mL sterile distilled water taken in test tubes and was mixed well. The solution (1 mL) was then transferred to test tubes with 9 mL sterile water to obtain 10^{-1} dilution. From this suspension, it was further diluted up to 10^{-4}, such

that the spore concentration was as low as four to five spores when observed in petri plate under low power microscope (100 X magnification).

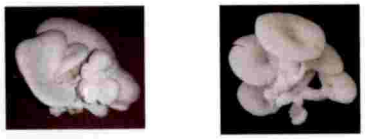
Plain agar of 2 per cent was prepared and autoclaved at 121°C and 1.05 kg cm^{-1} pressure for 15 min. The diluted spore suspension (1 mL) was pipetted out to the centre of sterile petri plate. A thin layer of plain agar was then poured into petri plate and was uniformly mixed. The spores were marked under low power microscope and were allowed to germinate for two days. The marked germinated spores were then picked by a sterile inoculation needle and were allowed to grow in PDPA media at 26 ± 2°C.

3.4.2 Cross Breeding of Monokaryotic Cultures

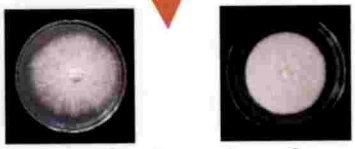
Single spore cultures obtained were observed for its mycelial appearance and radial growth rate. Among the cultures, fast growing, dense single spore cultures were selected from each strain. All the other slow growing and fluffy cultures were screened out.

The monosporous cultures of parental isolates were then tested for their compatibility by growing in petri plates containing PDPA media. Five mm discs of 10 day old cultures of monokaryotic mycelia were placed 2 cm apart in a 9 cm diameter petri plate containing PDPA media and was incubated at 25°C for seven days. The single spore cultures of isolate 1 were tested for compatibility in every possible combination with isolate 2 and isolate 3 separately. Periodical observation was also taken on the mating behaviour of the monokaryotic mycelium.

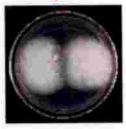
Formation of thick strand at the interaction zone of the monokaryotic culture implies the successful mating. Ten such compatible mating pairs were selected based on the prominent interaction and formation of thick tuft in the contact zone. A small portion of mycelium from the junction was cut and observed under microscope for clamp connection. Pairings were confirmed as compatible mating if clamp connections could be observed on the hyphae



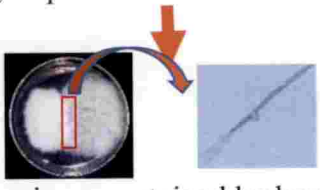
Isolation of single spores from desirable sporocarps



Culturing of spore and selection of ten fast growing single spore culture

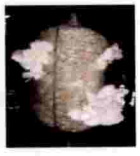


Dual culture of single spore culture of two different *Pleurotus* species



Selection of ten compatible pairs, accertained by barrage formation at the confluence region and presence of clamp connection

Culturing of dikaryotic mycelium from the confluence region, mother spawn production and preparation of mushroom beds



Selection of high yielding hybrids with blended characters

Tissue culturing from the selected sporocarp and repeating the cultivation upto three generation

Selection of stable hybrids with desired characters from third generation

Figure 2. Steps for hybridisation by crossing single spore culture of species of *Pleurotus*

(Anitha, 1998). Then a culture disc of 5 mm size was cut off from the confluence region on the petri plate and sub-cultured to PDPA plate. It was incubated for five to seven days at 26±2°C.

The heterokaryotic mycelia thus produced were screened; fast growing and thick stranded cultures were selected. These were brought to mother spawn production as described in 3.1.

3.4.3 Comparative Performance of Hybrids with Parents

The experiment was laid out in CRD with five treatments. Four replications were maintained for every treatment. Each replication denoted five mushroom beds. The crop was laid during November 2018 and the weather data is given in Appendix IV.

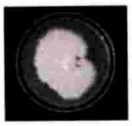
After twenty days of spawn inoculation, the white thick spawn produced was subjected to cultivation trial using paddy straw as substrate as described in 3.1. Observations on the fruiting behaviour of each dikaryon were recorded as in 3.3.

The better hybrids were subjected to generation studies (upto three generation) and the stable hybrid from each crossing *viz.,* isolate 1 x isolate 2 and isolate 1 x isolate 3 were selected for the comparative studies (Figure 2). Observations were recorded as per 3.3 and the data was analysed statistically.

3.5 STUDIES ON STRAIN IMPROVEMENT BY GAMMA IRRADIATION

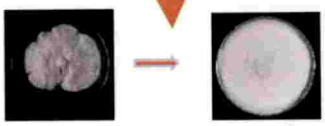
The experiment was laid out in CRD with nine treatments. The crop was laid during December 2018 and the weather data is given in Appendix IV.

A Co-60 irradiator (Gamma cell-5000) with a dose rate of 9.779 kGy h^{-1}, at Radio Tracer Laboratory of Kerala Agricultural University, Vellanikkara, Thrissur was used for gamma irradiating the isolates. Pure cultures of the isolates were grown in PDPA media and incubated at laboratory condition (26 ± 2°C). On



Pure culturing of *Pleurotus* spp. in PDPA media

Gamma irradiation at 20 Gy and 25 Gy



Separately culturing ten mycelial bits from periphery of each gamma irradiated culture

Mother spawn production followed by mushroom bed preparation

Screening for improved growth attributes and their selection

Tissue culturing of sporocarp and further stability studies by laying mushroom bed for three generation

Selection of stable gamma induced mutants and tissue culturing of sporocarp

Comparative study of the stable mutant with *Pleurotus* spp.

Figure 3. Steps for improving the strain by gamma irradiation of species of *Pleurotus*

the seventh day of inoculation, each culture was exposed to gamma irradiation at doses 20 and 25 Gy (Anitha, 1998).

Ten random mycelial bits were taken aseptically from the periphery of each irradiated cultures. The bits were separately cultured in petri plates and spawn was prepared in paddy grains as per 3.1. Beds were laid using paddy straw as substrate (3.1) at the rate of four replication beds per random culture disc of each treatment along with the control. Observations *viz.* days taken for spawn run, primordial initiation, crop period, total yield, disease and pest incidence etc. were recorded. The cultures exhibiting superior qualities were studied further for three generations to get the consistent results. The mutants with improved growth attributes and stability in characters were compared to their parents (Figure 3) and the observations were recorded as per 3.3.

3.6 COMPARISON OF THE BEST CULTURES

3.6.1 Yield

The improved cultures from the above experiments, *viz.,* 3.3, 3.4 and 3.5, were selected and brought to mushroom bed preparation along with the native isolates (parents). The crop was laid out during March, 2019 with four replication beds per treatment and the weather data is given in Appendix IV. Based on the observations, the improved strains were selected.

3.6.2 Proximate Constituents of Species of *Pleurotus*

The sporocarps from the best cultures obtained from 3.6.1 was analysed for the proximate contents.

3.6.2.1 Estimation of Moisture Content

Fresh mushroom samples harvested were weighed (w_1) and oven dried at 55 °C. The difference in fresh and dry weight (w_2) was recorded. It was expressed in percentage to calculate the moisture per cent (Geetha, 1993).

$$\text{Per cent moisture content (\%)} = \frac{w_1 - w_2}{w_1} \times 100$$

3.6.2.2 Estimation of Carbohydrate

Total carbohydrate content of species of *Pleurotus* was estimated using anthrone method (Aminoff *et al.*, 1970). Homogenized dried sample (100 mg) was weighed out in a clean boiling tube and was hydrolysed with 5 mL of 2.5 N HCl. The tube was kept in boiling water bath for 2-3 h. The hydrolysate was cooled to room temperature. Then powdered sodium carbonate was added slowly with constant shaking till effervescence ceased. The mixture was transferred to 100 mL volumetric flask along with distilled water. The volume was made up to 100 mL with the washings of the tube. It was thoroughly mixed and 10 mL of this solution was centrifuged at 5000 rpm for 10 min.

From the supernatant, aliquots were pipetted out at different volume *viz.*, 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL to test tubes and were made up to 1.0 mL by adding distilled water. To this, 4.0 mL of anthrone reagent was added and kept in boiling water bath for 10 min. It was cooled rapidly under running water and absorbance was read at 630 nm in spectrometer (Systronic UV-VIS Spectrophotometer). To prepare anthrone reagent, 200 mg of anthrone was dissolved in 100 mL concentrated sulphuric acid. For carbohydrate estimation, it was prepared fresh and chilled for 2 h before use.

3.6.2.3 Estimation of Crude Fibre

Powdered mushroom sample (2 g) was weighed out in a beaker. After adding 100 mL of 1.25 % sulphuric acid, it was boiled with constant stirring. The digested sample was strained through a muslin cloth and the residue was washed with boiling water to a beaker until washings were no longer acidic. The sample was boiled with 100 mL of 1.25 % sodium hydroxide solution for 30 min. It was again strained and washed with boiling water until the washings were not alkaline as mentioned earlier. For quantitative determinations, the residue was transferred

to a clean and dried crucible. The residue was weighed to determine the percentage of crude fibre.

The sample was then washed with 25 mL of 1.25 % boiling sulphuric acid, 50 mL of water and 25 mL of alcohol. The residue was transferred to pre-weighed silica crucible (w_1) and dried at 130 ± 15°C, till a constant weight was obtained. The dish was again weighed after cooling (w_2). The residue was further dried at 600 ±15 °C for 30 min, cooled and reweighed (w_3).

$$\text{Per cent crude fibre (\%)} = \frac{(w_2 - w_2) - (w_3 - w_1)}{\text{Weight of the sample}} \times 100$$

3.6.2.4 Estimation of Protein

Protein content of species of *Pleurotus* was estimated by Bradford's colorimetric method (Bradford, 1976). Dried sample (500 mg) was homogenized with 5 mL of potassium phosphate buffer and was centrifuged at 3000 rpm at 4° C for 15 min. The supernatant was collected in a volumetric flask (50 mL) and the volume was made up with phosphate buffer. The aliquot (1 mL) was transferred to centrifuge tube and 1 mL 20 % TCA (2,2,2- trichloroacetic acid) was added. After 30 minutes, it was centrifuged at 5000 rpm for 30 min. The supernatant was discarded and the pellet was washed twice with acetone. Each time, it was centrifuged and the supernatant was discarded. The pellet was dissolved in 2 mL 0.1N NaOH and 1.0 mL aliquot of this solution was used for the estimation.

Aliquots were pipetted out at different volume *viz.*, 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL to test tubes and were made up to 1.0 mL by adding phosphate buffer. To the aliquot, 5 mL dye solution (Bradford reagent) was added and mixed well. The absorbance of blue colour was read at 595 nm in spectrophotometer (Systronic UV-VIS Spectrophotometer 118) against reagent blank. The protein content was calculated from the standard curve and expressed as mg g^{-1} of the sample.

3.7 INCIDENCE OF PESTS AND DISEASES

The mushroom beds were periodically observed for the incidence of pests and diseases. The pests and the causal organism of the diseases were identified. The contaminated and pest infested beds were removed from the cropping room of mushroom house to prevent further spread.

3.8 RAPD ANALYSIS

RAPD profiling of improved strains along with the control was carried out at Advanced Research Centre for Plant Disease Diagnosis (ARCPDD), Department of Plant Pathology. RAPD of the improved strains was done using five random primers to calculate the similarity and polymorphism matrix. The procedure to conduct RAPD is elaborated below.

3.8.1 Isolation of Genomic DNA

The isolates along with their hybrids and improved mutants were cultured in petri plates containing PDPA media and incubated at 26 $\pm$ 2 ° C for seven days. Four discs of 5 mm size were cut with a sterile cork borer from the periphery of the mycelial culture. Discs were inoculated into 250 mL conical flask containing 100 mL of PDPA broth and incubated at 26 ± 2°C for 7 - 9 days. Once the mycelial bits colonized the surface of the flask, the mycelia were harvested from the broth by draining through a funnel containing Whatman No. 1 filterpaper.

Good quality genomic DNA was isolated from the improved strains and the parental isolates by using DNeasy plant mini kit (Qiagens). Samples ($\leq$100 mg wet weight or $\leq$20 mg lyophilized tissue) were disrupted in TissueRuptor®, the TissueLyser II or a mortar and pestle with 400 µL buffer AP1 and 4 µL RNase A. It was vortexed and incubated for 10 min at 65°C. Tube was inverted 2–3 times during incubation. Buffer P3 (130 µL) was added and incubated for 5 min on ice after mixing. The lysate was centrifuged 5 min at 14,000 rpm and was pipetted out into a QIA shredder spin column placed in a 2 mL collection tube.

After centrifuging for 2 min at 14,000 rpm, the flow through was transferred into a new tube without disturbing the pellet if present. Buffer AW1 was added (1.5 times volumes) and mixed by pipetting. The mixture (650 µL) was transferred into a DNeasy Mini spin column placed in a 2 mL collection tube. After centrifuging for 1 min at 8000 rpm, the flow-through was discarded. The spin column was placed into a new 2 mL collection tube and 500 µL buffer AW2 was added. It was centrifuged for 1 min at 8000 rpm and the flow-through was discarded. The procedure of adding 500 µL buffer AW2 and centrifugation was repeated. Note: The spin column from the collection tube was carefully removed so that the column did not come into contact with the flow-through. The spin column was placed to a new 1.5 mL or 2 mL microcentrifuge tube and 100 µL buffer AE was added for elution. It was incubated for 5 min at room temperature (15-25°C) and centrifuged for 1 min at 8000 rpm. The step with elution buffer was repeated and the extract was transferred to a 1.5 µL new centrifuge tube.

3.8.2 PCR Amplification

The DNA isolated was subjected to PCR amplification using five decamer primers selected based on the earlier reports of Agarwal *et al* (2013). The primers tested were OPT 5 (GGGTTTGGCA), OPS 5 (TTTGGGGCCT), OPZ 10 (CCGACAAACC), OPA 10 (GTGATCGCAG) and OPD 7 (TTGGCACGGG) The synthesized primers being in powdered form were diluted to a final concentration of 10 pmol with DEPEC water prior to its use.

The PCR amplification reactions were carried out in a 25 µL reaction mixture containing 1 µL of 2 U of Taq DNA polymerase, 0.5 µL of 2.5mM $MgCl_2$, 2 µL of 2.5 mM dNTPs, 10 X PCR buffer 2.5 µL (100 mM Tris Hcl), 100 ng DNA and 10pmol of primer. The reaction mixture was run in a thermocycler (Veriti 96 well Thermal cycler, Applied Biosystems) under the specified conditions. The amplification was conducted by initial denaturation of template DNA at 95 °C for 4 min followed by 35 cycles of DNA denaturation at

94 °C for 1 min, primer annealing at 32°C for 45 sec, initial primer extension at 72 °C for 2 min, followed by a final extension at 72 °C for 10 min.

3.8.3 Agarose Gel Electrophoresis of PCR Products

Agarose gel of 1.2 per cent was prepared in 0.5X TAE buffer containing 0.5µg ml^{-1} ethidium bromide (EtBr). Two µL of 6X loading dye was mixed with 10 µL of PCR products, loaded and electrophoresis was performed at 75V cm^{-1} power supply with 0.5X TAE as electrophoresis buffer for about 1-2 h. The molecular standards used was 100 bp DNA ladder (GeNei). The gels were then visualized and image was documented using Gel documentation system (Bio-Rad Gel DOC ™ XR+) under UV light.

The RAPD profiles were analyzed based on the presence or absence of individual RAPD bands. The bivariate data were analysed to generate Jaccard's similarity coefficient. Jaccard's similarity coefficient values for each pair wise comparison between accessions were calculated and similarity coefficient matrix was constructed. The matrix was used for grouping the mushroom samples based on the dendrogram constructed by UPGMA (Unweighed Pair Group Method with Arithmetic averages). All the numerical analysis was conducted using the software SPSS.

3.9 STATISTICAL ANALYSIS

The data obtained from the experiments were subjected to analysis of variance (ANOVA). Critical difference (CD) was calculated at 5 per cent level of significance and used for comparison of difference between the treatment means. Standard error mean and standard deviation of observations were also determined. Statistical analysis of the data was done using both WASP 2.0 and OPSTAT softwares.

Results

4. RESULTS

Pleurotus commonly known as Oyster mushroom is the second most commercially cultivated mushroom in world as well as in India. New strains of *Pleurotus* spp. with superior quality are being identified at different places and this requires further scientific studies before releasing for commercialization. Better strains from the existing oyster mushrooms could be developed by various methods like introduction, selection, hybridisation, mutation etc. In this regard, the present study was undertaken in three native isolates *viz.*, *P. djamor, P. florida* and isolate with 80 per cent similarity to *P. florida,* collected from Mushroom Unit, Instructional farm, College of Agriculture, Vellayani. Species level confirmation of isolate 3 was done at molecular level ITS sequencing and was confirmed as *P. ostreatus* (Appendix v). Hybridisation and mutation studies were undertaken in these isolates with the objective to develop new promising strains.

4.1 MORPHOLOGICAL CHARACTERISATION OF SPOROCARP OF NATIVE ISOLATES

4.1.1 Macroscopical Observations

The sporocarps from the species of *Pleurotus* viz., *P. djamor, P. florida* and *P. ostreatus* were studied based on the pileus colour, pileus dimension, stipe length, sporocarp weight and texture. The descriptions on macroscopical characteristics are given in table 1.

P. djamor produced pink coloured leathery sporocarps with smaller pileus. It was characterized by spathulate to flabelliform pileus with entire margin (Plate 1). The pileus dimension of *P. djamor* was 4.80 cm × 6.57 cm. Usually the sporocarp was without stipe and if present, it was shortly stiped with an average length of 0.92 cm. The average fresh sporocarp weighed 6.03 g and the average number of gills per cm of the sporocarp from the margin was 17.87. *P. djamor* attained harvesting maturity in three days from pinhead emergence. Pinheads

produced were pink in colour with short stipe. On maturity, the colour faded and the stipe became non prominent (Plate 2).

P. florida produced white coloured delicate and decurrent sporocarps with entire, enrolled type pileus margin of comparatively larger pileus with length 6.46 cm and breadth 7.58 cm (Plate 3). Stout long stipe (3.33 cm) was recorded for sporocarp of *P. florida*. The average weight of individual sporocarp recorded was 11.47 g and it had 11.40 gills per cm of the pileus. Similar to *P. djamor*, *P. florida* also needed three days to attain harvesting maturity from pinhead emergence (Plate 4). The pinheads were whitish and produced in bunches. On attaining maturity, the circular pileus became spathulate in shape and the stipe got elongated and bulged.

P. ostreatus produced greyish white coloured sporocarps with entire margin (Plate 5). Among the isolates, pileus dimension was comparatively larger for *P. ostreatus*, with 7.40 cm length and 8.27 cm breadth. Average sporocarp weight of sporocarp was 10.72 g and number of gills on margin of the pileus was 13.73 per cm. The gills were arranged falcate decurrently to the stipe having 5.58 cm. *P. ostreatus* attained the harvesting stage from pinhead emergence in four days (Plate 6). The pinheads were produced in larger bunches compared to *P. djamor* and *P. florida*. The light blue colour at the initial stages of sporocarp development turned to greyish white or white colour on attaining the harvesting maturity.

4.1.2 Microscopical Observations

Microscopical observations of hyphae, spores and cystidia of species of *Pleurotus* were recorded (Table 2). Hyphae of species of *Pleurotus* were septate, branched and hyaline with clamp connections (Plate 7). The width of the hyphae did not vary significantly among each other and it ranged from 1.5-4.5 µm. The basidial dimension (length and width) of *P. djamor*, *P. florida* and *P. ostreatus* were more or less similar with size 22-28 µm × 4-6 µm, 24-31 µm × 5-9 µm and

Table 1. Biometric observations of sporocarp of species of *Pleurotus* cultivated in paddy straw substrate

Species of *Pleurotus*	Pileus length (cm)	Pileus breadth (cm)	Stipe length (cm)	Sporocarp weight (g-fresh weight)	Gills (No. cm^{-1})	Colour	Texture
*P. djamor**	4.80±0.60	6.57±0.65	0.92±0.16	6.03±1.15	17.87±0.91	Pink	Leathery
*P. florida**	6.46±1.11	7.58±1.45	3.33±0.44	11.47±2.10	11.40±0.51	White	Delicate
*P. ostreatus***	7.40±1.23	8.27±0.69	5.58±0.66	10.72±2.03	13.73±0.59	Greyish white	Delicate

Values were recorded on 3rd * and 4th day** after primordial initiation

Values are mean ± SD of 15 sporocarps

Table 2. Microscopical observations of species of *Pleurotus* cultivated in paddy straw substrate

Species of *Pleurotus*	Mycelial width (µm)	Basidia (µm) (l × b)	Basidiospores (µm) (l × b)	Cheilocystidia (µm) (l × b)
P. djamor	1.5-4.5	22-28 × 4-6	8-12 × 3-5	25-33 × 6-9
P. florida	1.9-4.2	24-31 × 5-9	8-13 × 3-5	31-45 × 7-8
P. ostreatus	1.8-4.3	22-28 × 8-10	5-7 × 2-3	34-40 × 7-10

Values are range of 30 observations

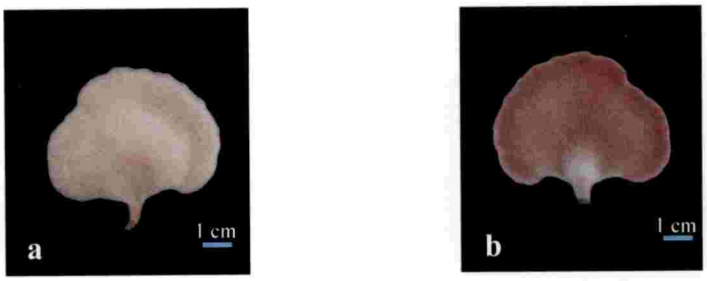


Plate 1. Sporocarp of *P. djamor* (pink and spathulate shaped pileus with short stipe); a. Upper side; b. Lower side

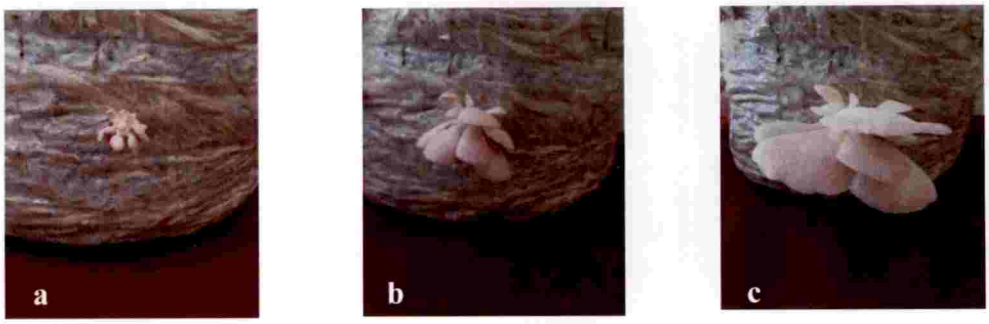


Plate 2. Development stages of *P. djamor* from pinheads to harvesting maturity; a. Day 1-pinheads; b. Day 2- Elongation of stipe; c. Day 3- Enlargement of pileus

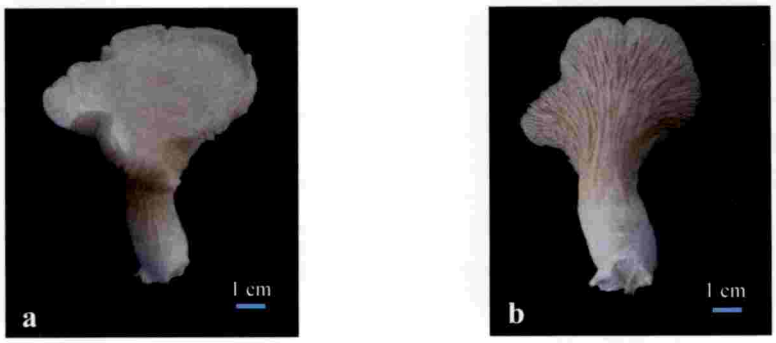


Plate 3. Sporocarp of *P. florida* (white and spathulate shaped pileus with stout stipe); a. Upper side; b. Lower side

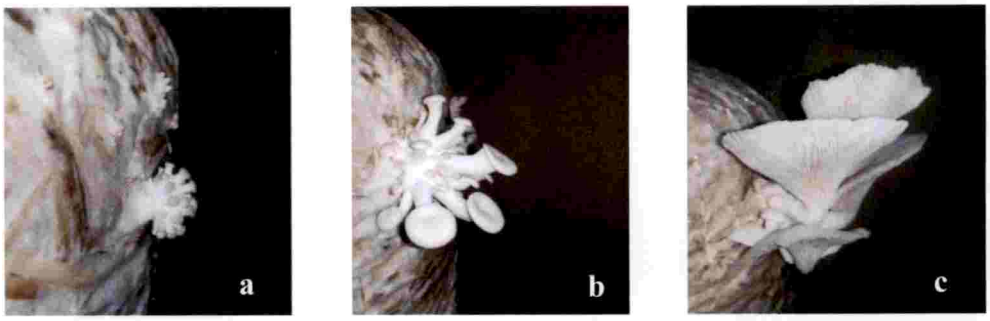


Plate 4. Development stages of *P. florida* from pinheads to harvesting maturity; a. Day 1-pinheads; b. Day 2- Elongation of stipe; c. Day 3- Enlargement of pileus

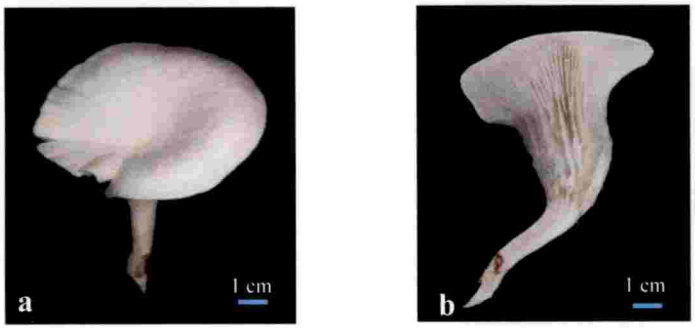


Plate 5. Sporocarp of *P. ostreatus* (greyish white pileus with long stipe and falcate decurrent gills); a. Upper side; b. Lower side

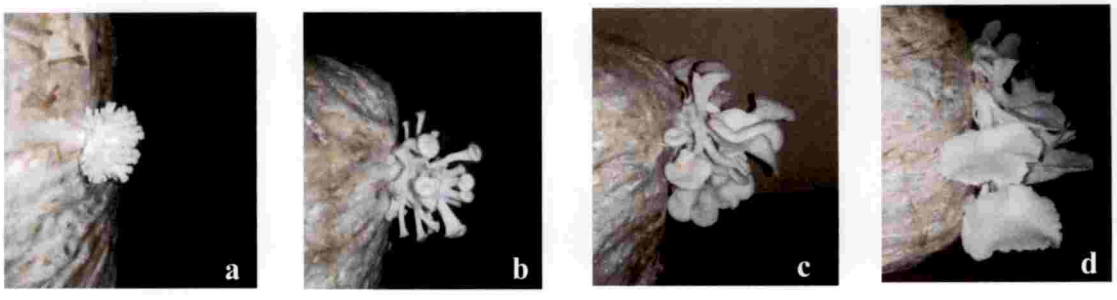


Plate 6. Development stages of *P. ostreatus* from pinheads to harvesting maturity a. Day 1-Greyish white pinheads; b. Day 2- Elongation of stipe; c. Day 3- Enlargement of pileus; d. Day 4- Enlargement and flattening of pileus

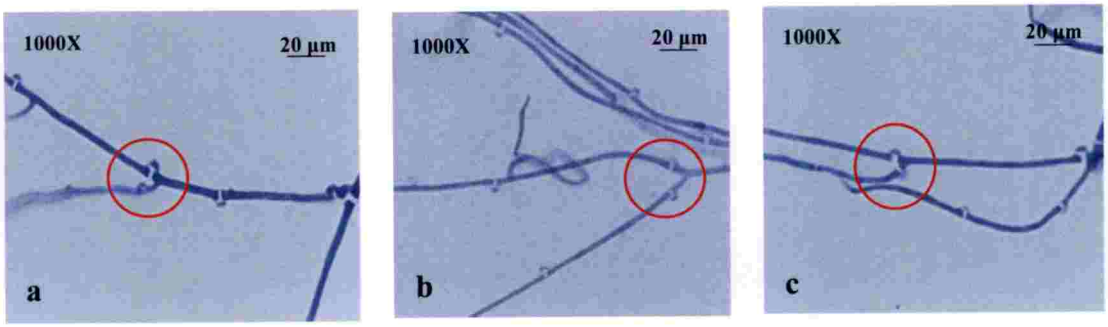


Plate 7. Mycelium of species of *Pleurotus* with clamp connections; a. *P. djamor;* b. *P. florida;* c. *P. ostreatus*

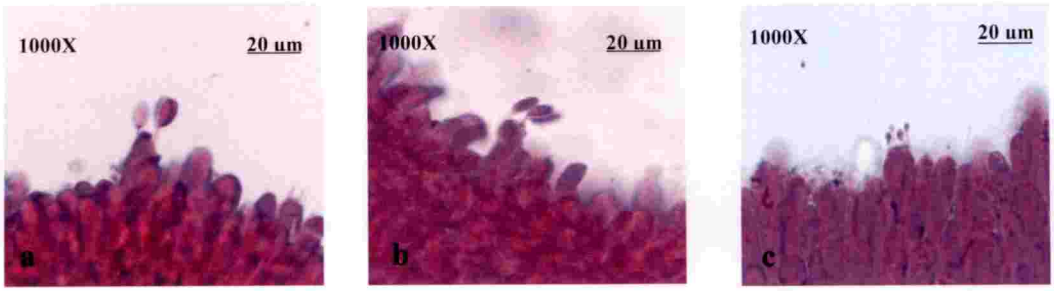


Plate 8. Clavate shaped tetrasterigmatic basidia of species of *Pleurotus* bearing basidiospores; a. *P. djamor;* b. *P. florida;* c. *P. ostreatus*

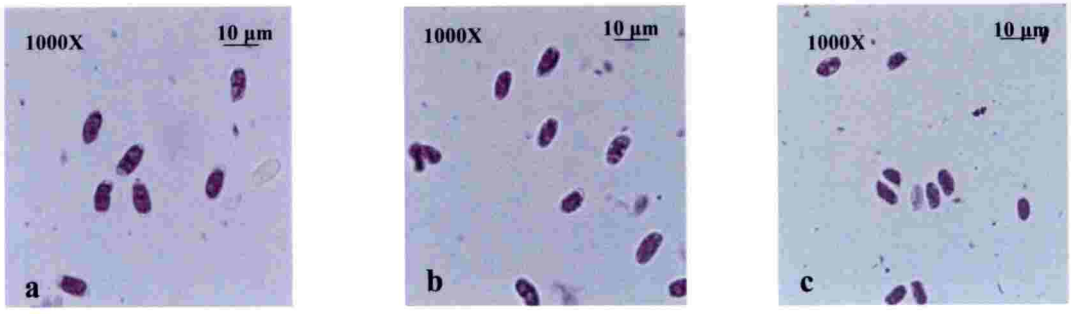


Plate 9. Cylindrical shaped basidiospores of species of *Pleurotus;* a. *P. djamor;* b. *P. florida;* c. *P. ostreatus* (smaller size)

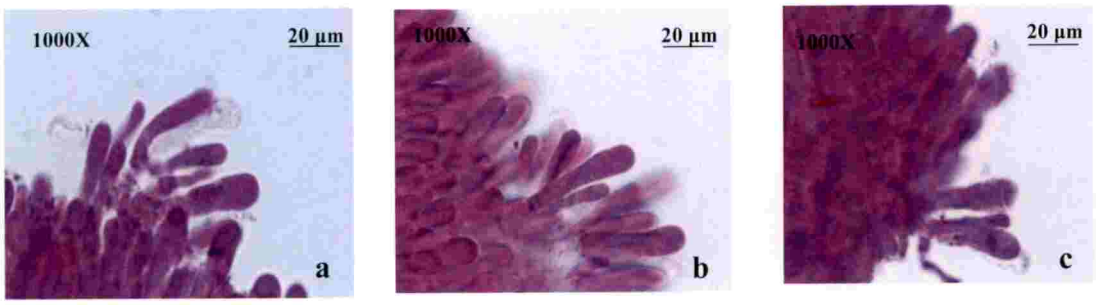


Plate 10. Subventricose to clavate shaped cystidia of species of *Pleurotus*; a. *P. djamor;* b. *P. florida;* c. *P. ostreatus*

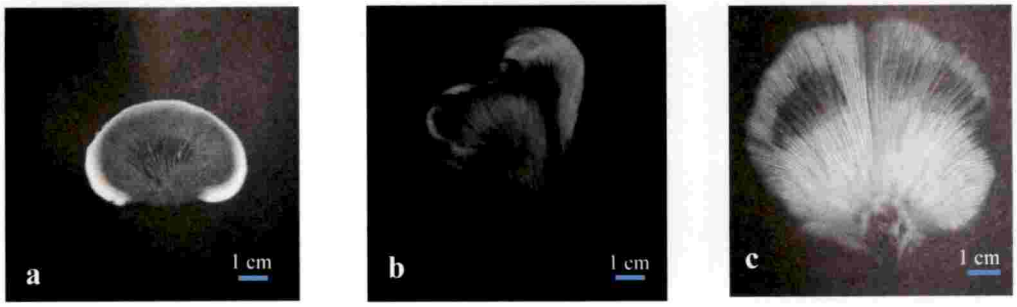


Plate 11. Spore print of species of *Pleurotus* indicating their gill arrangements; a. *P. djamor;* b. *P. florida;* c. *P. ostreatus*

22-28 µm × 8-10 µm respectively. The basidia of all the three species of *Pleurotus* had clavate shape and with tetrasterigmata producing four basidiospores (Plate 8). Basidiospores of species of *Pleurotus* were hyaline and cylindrical in shape (Plate 9). Basidiospores of *P. djamor* and *P. florida* recorded 8-12 µm in length and 3-5 µm width whereas, *P. ostreatus* produced spores with dimension 5-7 µm × 2-3 µm. All the three species of *Pleurotus* had relatively large sized sterile structures called cheilocystidia present between clusters of basidia (Plate 10). Comparatively smaller sized cheilocystidia were observed in *P. djamor* with a dimension of 25-33 µm length and 6-9 µm width. The cheilocystidia of all the three species of *Pleurotus* had subventricose to clavate shape. Spore print of the *P. djamor* was light pink in colour which later changed to creamish white. White spore print was observed in *P. florida* and *P. ostreatus* (Plate 11).

4.2 RE-ISOLATION AND PURE CULTURING OF SELECTED ISOLATES AND STUDYING MORPHOLOGICAL CHARACTERS OF THE CULTURE

Partially matured healthy, pest and disease free sporocarps of the three *Pleurotus* spp. were cultured on PDPA medium by tissue culture technique. New mycelial growth from the tissue was then pure cultured (Plate 12, plate 13) and morphological studies were done (Table 3).

Thick cream coloured cottony strand with concentric pattern was produced by *P. djamor*. Whereas white fluffy mycelium with even margin and thick stranded greyish white mycelium with radiating margin was observed in *P. florida* and *P. ostreatus* respectively. Rate of mycelial growth of *P. florida* (10.44 mm day^{-1}) was significantly higher and it attained complete growth in 9 cm diameter petri plate in minimum days (nine days). It was followed by *P. ostreatus* (8.90 mm day^{-1}) and *P. djamor* (7.58 mm day^{-1}) which completed its growth in petri plate in 10 and 12 days respectively.

Table 3. Cultural characters and mycelial growth rate of species of *Pleurotus* in PDPA medium

Species of *Pleurotus*	Mycelial growth pattern	Colour of mycelia	Rate of mycelial growth (mm day^{-1}) *	Days taken for complete growth in 9 cm petridish
P. djamor	Thick cottony strand with concentric pattern	Cream	7.58 ± 0.38^{c}	12
P. florida	Fluffy with even margin	White	10.44 ± 0.74^{a}	9
P. ostreatus	Thick strand with radiating margin	Greyish white	8.90 ± 0.68^{b}	10
CD(0.05)			0.857	
SE(m) ±			0.275	

Values are mean ± SD of five replications

Values followed by similar superscripts are not significantly different at 5 % level

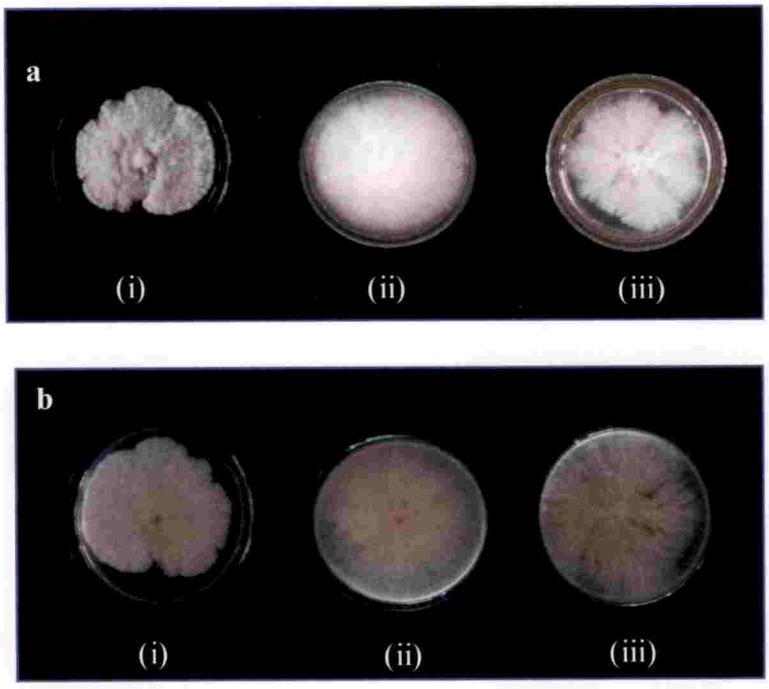


Plate 12. Radial growth of species of *Pleurotus* in PDPA medium on 9th day of inoculation; a. Upper side; b. Rear side (i) *P. djamor* with thick cream coloured mycelium; (ii) *P. florida* with faster growth rate; (iii) *P. ostreatus* with radiating margin

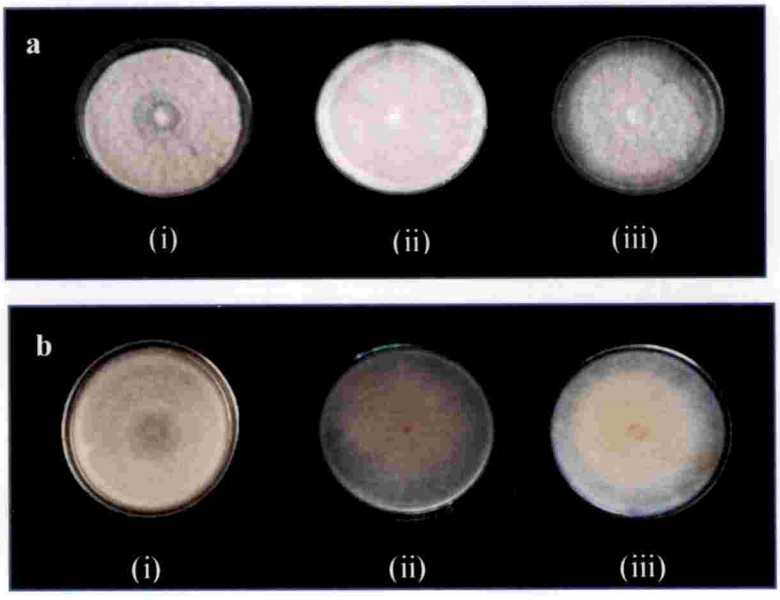


Plate 13. Cultural characters of species of *Pleurotus* in PDPA medium; a. Upper side; b. Rear side (i) *P. djamor* with concentric pattern; (ii) *P. florida* with white fluffy mycelium; (iii) *P. ostreatus* with greyish white mycelium

4.3 STUDIES ON STRAIN IMPROVEMENT BY HYBRIDISATION THROUGH SPAWN MIXING

Spawn of two different species were mixed and cultivation trials were conducted. Spawn mixed beds of *P. djamor* with *P. florida* and *P. ostreatus* were prepared and comparative performance of the treatments were analyzed (Table 4). Among the treatments, *P. djamor* completed its spawn run (9.75 days) significantly earlier than *P. djamor* + *P. florida* (12.25 days) and *P. djamor* + *P. ostreatus* (13.25 days) which were on par with each other. It was followed by *P. ostreatus* and *P. florida* (19 and 19.75 days respectively). Similarly, earliness in primordial initiation was observed in *P. djamor* (14.75 days) followed by *P. djamor* + *P. florida* (16 days) and *P. djamor* + *P. ostreatus* (16.35 days) which did not differ significantly with each other. *P. florida* and *P. ostreatus* recorded 25.75 and 24.25 days respectively for pinhead emergence.

Maximum number of sporocarps were produced in *P. ostreatus* (125.50), followed by *P. djamor* (97.75) and *P. florida* (85.50). The number of sporocarp produced was lowest for *P. djamor* + *P. florida* (57.50) and *P. djamor* + *P. ostreatus* (58.50).

The yield recorded was highest for *P. ostreatus* (828.25 g kg^{-1}) followed by *P. florida* (703.75 g kg^{-1}) and *P. djamor* (406.25 g kg^{-1}). Whereas significantly low yield was recorded in spawn mixed beds *viz.*, *P. djamor* + *P. florida* (235.75 g kg^{-1}) and *P. djamor* + *P. ostreatus* (258.25 g kg^{-1}). Both the spawn mixed beds produced sporocarps of their parents separately in the same bed (Plate 14 and plate 16). The crop period was also reduced significantly compared to their parents (Plate 15 and plate 17). *P. florida* and *P. ostreatus* had significantly longer duration (53.5 and 54.5 days) compared to *P. djamor*, *P. djamor* + *P. florida* and *P. djamor* + *P. ostreatus* (41.0, 33.24 and 30.75 days respectively).

Table 4. Comparative performance of species of *Pleurotus* and their mixed spawn cultivated in paddy straw substrate

Species of *Pleurotus* and their mixed spawn	Days taken for complete spawn run	Days taken for pinhead formation	Days taken for first harvest	Total yield* (g-fresh weight)	Crop period (days)	Sporocarp weight (g-fresh weight)	No. of sporocarp	BE (%)
P. djamor	9.75±0.50^{c}	14.75±0.50^{d}	17.75±0.50^{c}	406.25±15.84^{c}	41.00±0.82^{b}	6.03±1.15	97.75±8.34^{b}	40.62
P. florida	19.75±0.96^{a}	25.75±0.96^{a}	28.75±0.96^{a}	703.75±17.40^{b}	53.50±1.29^{a}	11.47±2.10	85.50±5.20^{b}	70.38
P. ostreatus	19.00±0.82^{a}	24.25±0.50^{b}	28.25±0.50^{a}	828.25±49.68^{a}	54.50±1.29^{a}	10.72±2.03	125.50±16.42^{a}	82.83
P. djamor + *P. florida*	12.25±0.50^{b}	16.00±0.82^{c}	19.00±0.82^{b}	235.75±12.92^{d}	33.24±1.91^{c}	6.03±1.15 8.15±0.53	57.50±9.04^{c}	23.58
P. djamor + *P. ostreatus*	13.25±0.96^{b}	16.35±0.82^{c}	19.35±0.82^{b}	258.25±32.64^{d}	30.75±2.63^{c}	6.03±1.15 7.93±0.70	58.50±7.77^{c}	25.83
CD (0.05)	1.178	1.128	1.128	44.366	2.597		15.334	
SE(m) ±	0.387	0.371	0.371	14.285	0.854		5.041	

* Total yield from three harvest

Values are mean ± SD of four replications

Values followed by similar superscripts are not significantly different at 5 % level

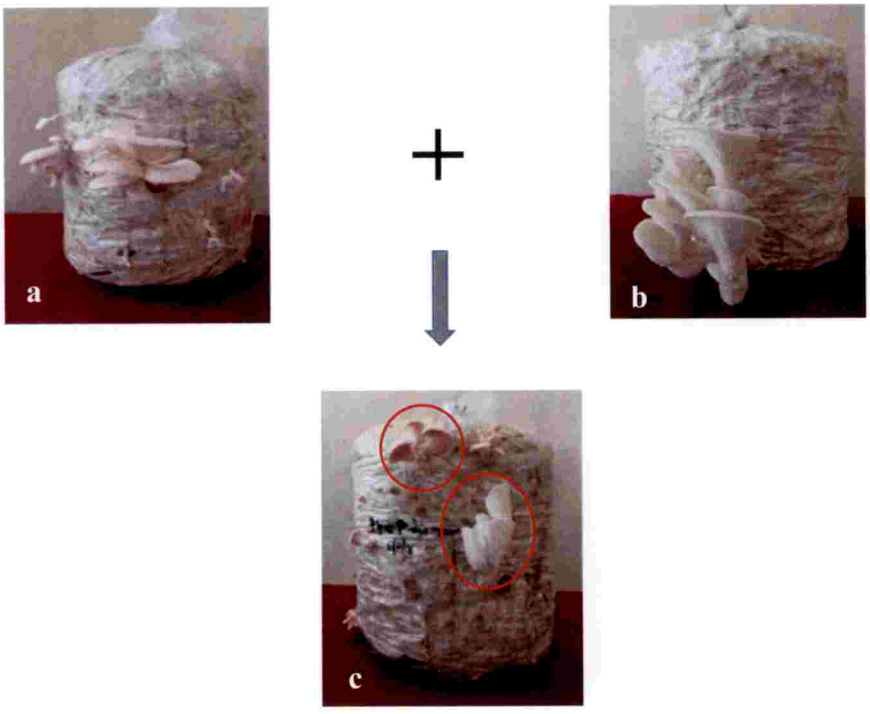


Plate 14. Production of fruiting bodies of both *P. djamor* and *P. florida* on their spawn mixed bed; a. *P. djamor*; b. *P. florida* c. *P. djamor* + *P. florida*

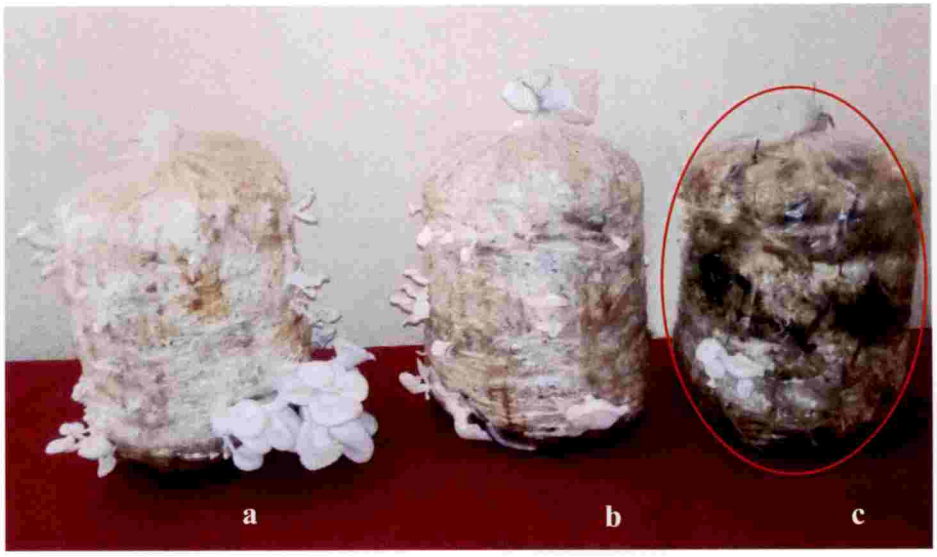


Plate 15. Reduced yield performance and crop period of mixed spawn of *P. djamor* and *P. florida* at 40 days after spawning compared to *P. djamor* and *P. florida*; a. *P. florida*; b. *P. djamor;* c. *P. djamor* + *P. florida*

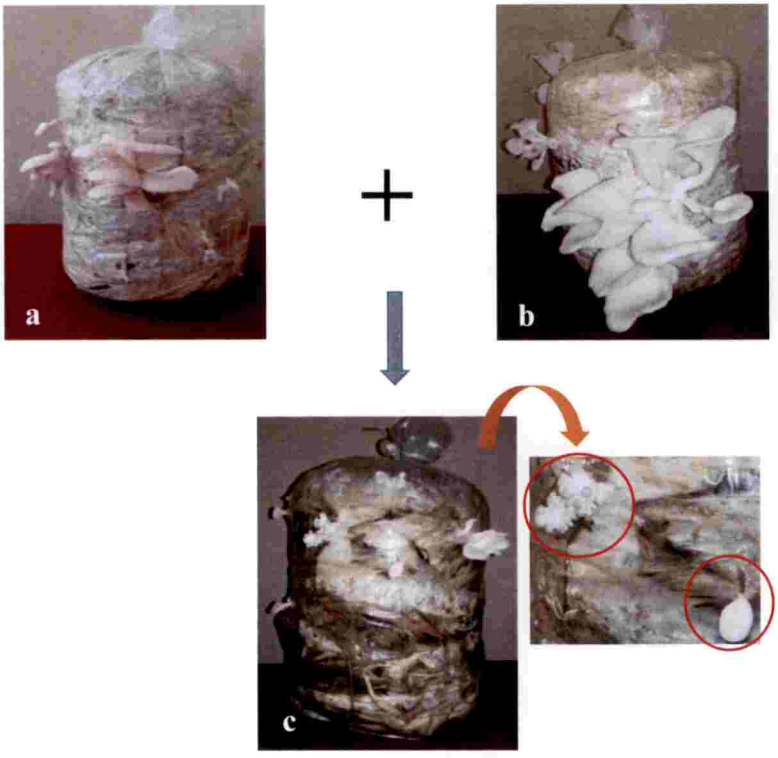


Plate 16. Production of fruiting bodies of both *P. djamor* and *P. ostreatus* on their spawn mixed bed; a. *P. djamor*; b. *P. ostreatus* c. *P. djamor* + *P. ostreatus*

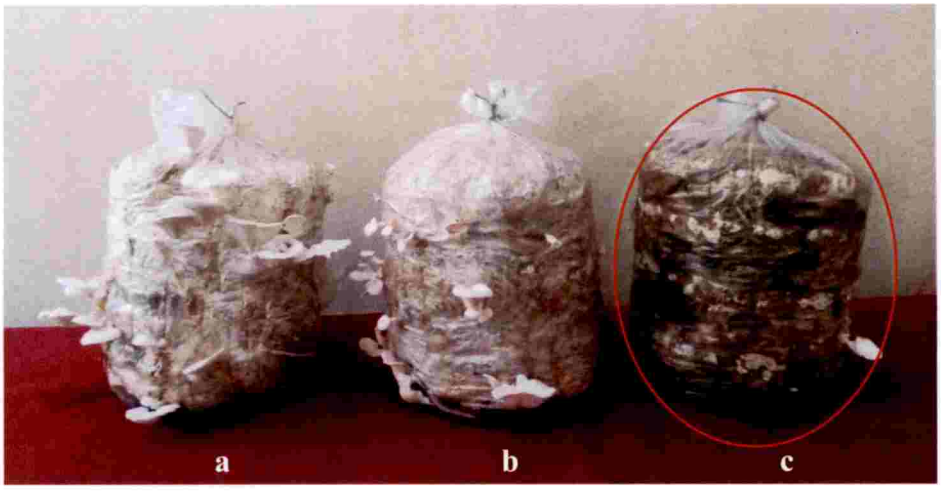


Plate 17. Reduced yield performance and crop period of mixed spawn of *P. djamor* and *P. ostreatus* at 40 days after spawning compared to *P. djamor* and *P. ostreatus*; a. *P. ostreatus*; b. *P. djamor;* c. *P. djamor* + *P. ostreatus*

4.4 STUDIES ON STRAIN IMPROVEMENT BY HYBRIDISATION THROUGH CROSSING OF SINGLE SPORE CULTURE

4.4.1 Preparation of Monosporous Culture

Spores were extracted from healthy medium matured sporocarp. Among 50 spores marked under microscope, early germinated 20 spores were allowed to grow in petri plates containing PDPA medium. Hybridisation between the isolated monosporous cultures was conducted as described in 3.4.2.

4.4.2 Pairing of Compatible Monosporous Cultures

Monosporous cultures was dual cultured in petri plates containing PDPA medium. Based on the overall growth performance of the homokaryons, 10 single spore cultures were selected from each isolates. These were dual cultured on PDPA medium and the compatability of the isolates were checked. Compatibility of monosporous cultures were identified by the formation of thick tuft at the confluence region (Plate 18, plate 19). It was further confirmed by the presence of clamp connection under microscope (Plate 20). From the dual culture plates of *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus*, 10 compatible mating pairs were selected. Mycelial bit from the confluence region was then cut and cultured in PDPA medium. Thick stranded fast growing six dikaryon cultures were selected for study and spawn production. Fully matured spawn was then used for mushroom bed preparation and the performance was recorded. Sporocarps with intermediate characters and increased yield was observed in mushroom beds prepared from two selected cultures of *P. djamor* x *P. florida* and three cultures of *P. djamor* x *P. ostreatus*. Hybrids were then subjected to stability studies for three generation and the best one hybrid from each of the above was selected.

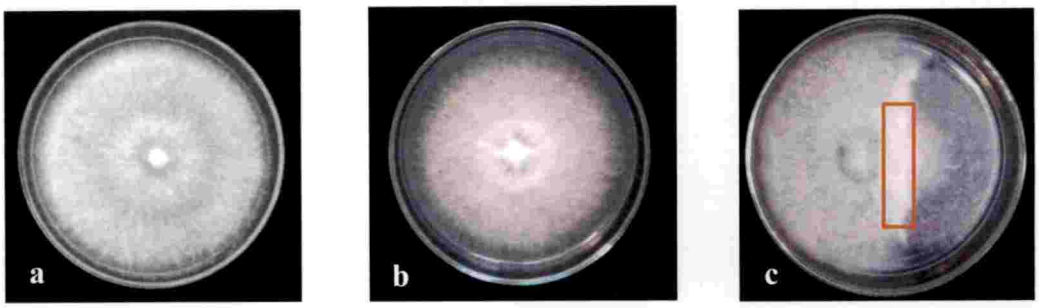


Plate 18. Single spore culture of *P. djamor*, *P. florida* and their dual culture; a. *P. djamor*; b. *P. florida*; c. Barrage formation in confluence region of *P. djamor* and *P. florida*

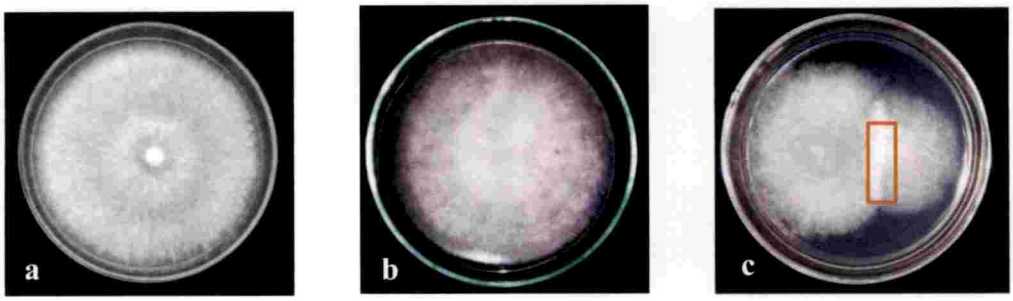


Plate 19. Single spore culture of *P. djamor*, *P. ostreatus* and their dual culture; a. *P. djamor*; b. *P. ostreatus*; c. Barrage formation in confluence region of *P. djamor* and *P. ostreatus*

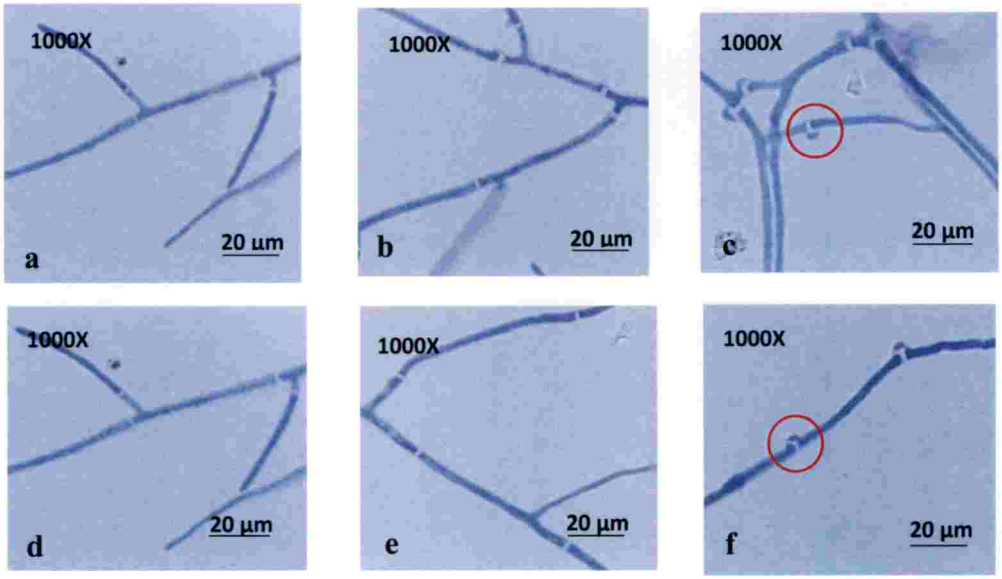


Plate 20. Mycelium of single spore culture of species of *Pleurotus* and their hybrids indicating absence/ presence of clamp connection; a. *P. djamor;* b. *P. florida*; c. *P. djamor* x *P. florida* d. *P. djamor;* e. *P. ostreatus;* f. *P. djamor* x *P. ostreatus*

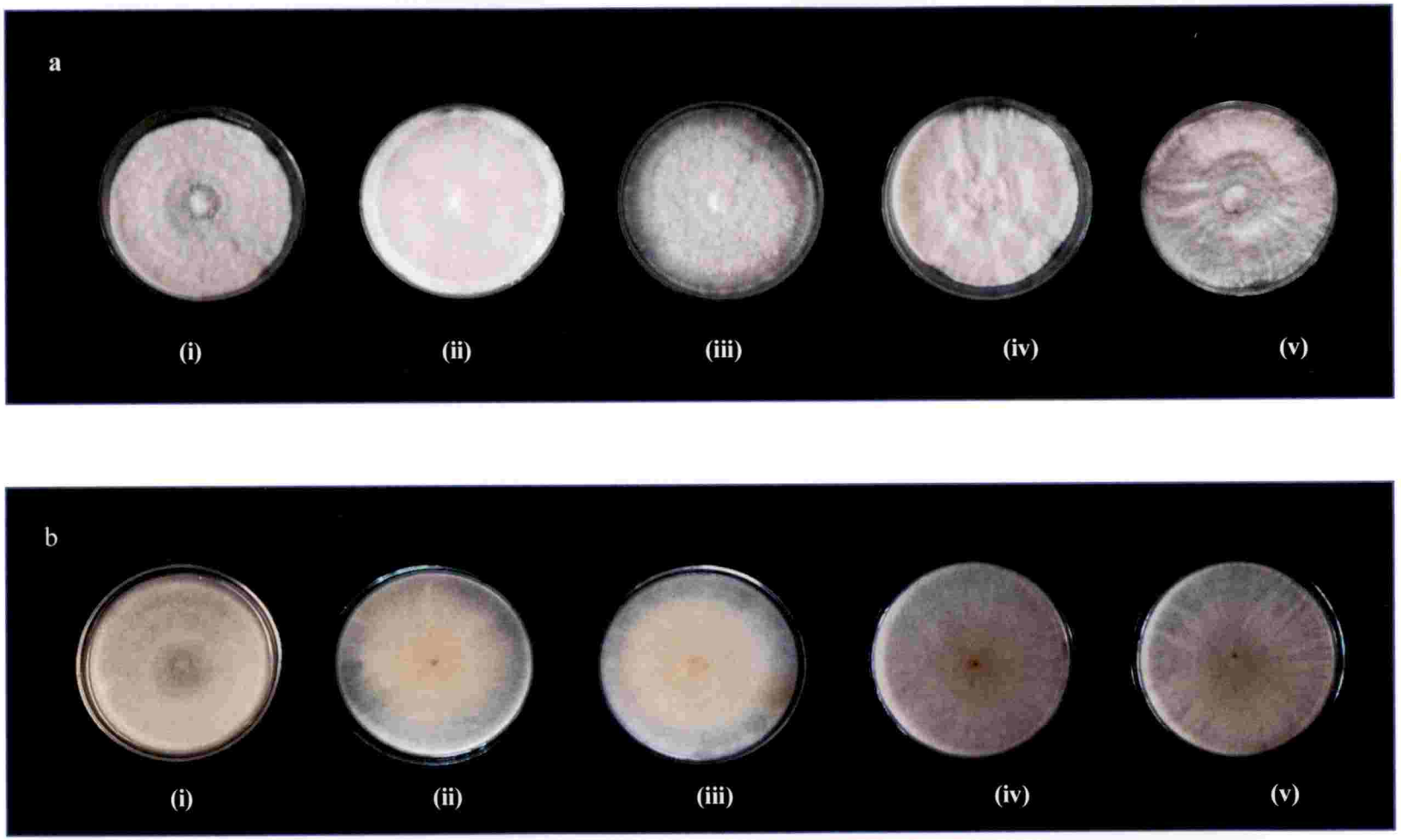


Plate 21. Colony morphology of species of *Pleurotus* and their hybrids in PDPA medium; a. Upper side; b. Rear side (i). *P. djamor* with concentric pattern; (ii). *P. florida* with white fluffy mycelium; (iii). *P. ostreatus* with greyish white mycelium; (iv). *P. djamor* x *P. florida* with cottony cream mycelium; (v). *P. djamor* x *P. ostreatus* with greyish white mycelium and radiating growth

4.4.3 Comparative Performance of Hybrids with Parents

Hybrids along with their parents were subjected to cultivation trials and the data were recorded (Table 5, plate 21). Observation on number of days needed for spawn production revealed that both the hybrids, *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus* was significantly superior which completed the spawn production in minimum number of days (8.75 and 8.50). This was followed by *P. djamor* which completed the spawn production in 15.75 days (Plate 22). Completion of mycelial run in spawn was delayed in *P. ostreatus* (20.50 days) and *P. florida* (20.75 days), which were on par with each other. The hybrids completed spawn run in mushroom bed in 7.75 days and was followed by *P. djamor*, *P. ostreatus* and *P. florida* (9.75, 19.00 and 19.75 days respectively). After spawning, the pinheads appeared first on *P. djamor* x *P. ostreatus* (12.25 days) and *P. djamor* x *P. florida* (13.00 days) which was significantly earlier than their parents (Plate 23). It was followed by *P. djamor* (14.75 days), *P. ostreatus* (24.25 days) and *P. florida* (25.75 days), which were significantly different from each other.

The BE of hybrids along with their parents were analyzed (Plate 24, plate 25). Maximum BE of 82.83 per cent was obtained from *P. ostreatus* which was followed by *P. florida* (70.38 %). Data revealed that the hybrid, *P. djamor* x *P. ostreatus* recorded BE of 60.33 per cent followed by *P. djamor* x *P. florida* (54.60 %) which was significantly superior to the parent, *P. djamor* (40.63 %). Among the *Pleurotus* spp. cultivated, average sporocarp weight was found maximum for *P. florida* (11.47 g) which was statistically on par with *P. ostreatus* (10.72 g). This was followed by the hybrid, *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus* (7.93 g and 7.69 g), which did not differ statistically with each other. Whereas *P. djamor* produced sporocarps with lowest average weight (6.03 g). Among the isolates, *P. ostreatus* produced significantly higher number of sporocarps (125.50) followed by the hybrid of *P. djamor* x *P. ostreatus* (102.75 g). Reduced crop period was recorded in *P. djamor* (41 days) which was statistically on par with *P. djamor* x *P. florida* (43.25 days) and *P. djamor* x

Table 5. Comparative performance of species of *Pleurotus* and their hybrids developed through hybridisation followed by three generation of selection in paddy straw substrate

Species of *Pleurotus* and their hybrids	Days taken for spawn production	Days taken for complete spawn run	Days taken for pinhead formation	Days taken for first harvest	Total yield* (g-fresh weight)	Crop period (days)	Sporocarp weight (g-fresh weight)	No. of sporocarp	BE (%)
P. djamor	15.75±0.50^{b}	9.75±0.50^{b}	14.75±0.50^{c}	17.75±0.50^{b}	406.25±15.84^{e}	41.00±0.82^{b}	6.03±1.15^{c}	97.75±8.34^{bc}	40.63
P. florida	20.75±0.96^{a}	19.75±0.95^{a}	25.75±0.96^{a}	28.75±0.96^{a}	703.75±17.40^{b}	53.50±1.29^{a}	11.47±2.10^{a}	85.50±5.20^{c}	70.38
P. ostreatus	20.50±0.58^{a}	19.00±0.82^{a}	24.25±0.50^{b}	28.25±0.50^{a}	828.25±49.68^{a}	54.50±1.29^{a}	10.72±2.03^{a}	125.50±16.42^{a}	82.83
P. djamor x *P. florida*	8.75±0.50^{c}	7.75±0.50^{c}	13.00±0.82^{d}	16.00±0.82^{c}	546.00±42.43^{d}	43.25±1.89^{b}	7.93±0.65^{b}	92.00±4.96^{bc}	54.60
P. djamor x *P. ostreatus*	8.50±0.58^{c}	7.75±0.50^{c}	12.25±0.50^{d}	15.25±0.50^{c}	603.25±27.97^{c}	43.50±2.64^{b}	7.69±0.81^{b}	102.75±5.50^{b}	60.33
CD (0.05)	1.128	1.039	1.039	1.039	50.919	2.597	1.081	13.826	
SE (m) ±	0.371	0.342	0.342	0.342	16.74	0.854	0.541	4.589	

* Total yield from three harvest

Values are mean ± SD of four replications

Values followed by similar superscripts are not significantly different at 5 % level

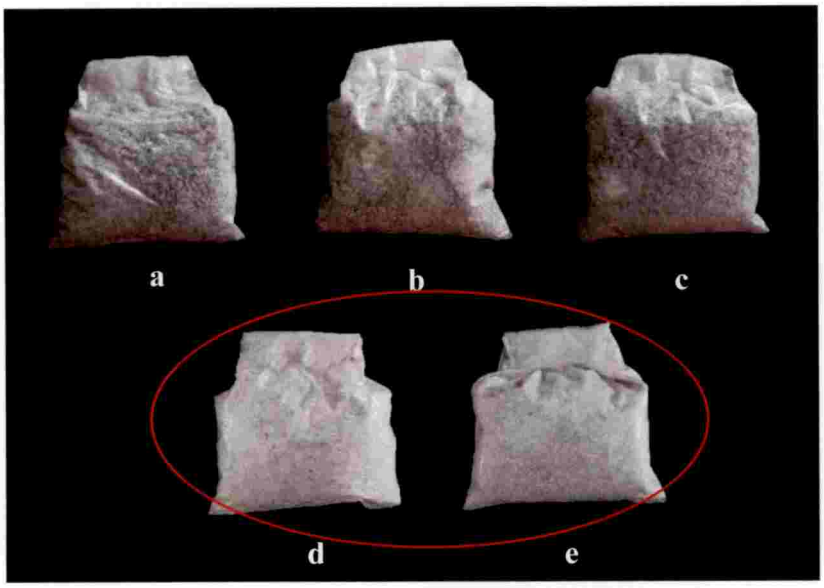


Plate 22. Earliness in spawn production of hybrids compared to their parental species of *Pleurotus* on 9th day of culture inoculation; a. *P. djamor*; b. *P. florida*; c. *P. ostreatus*; d. *P. djamor* x *P. florida*; e. *P. djamor* x *P. ostreatus*

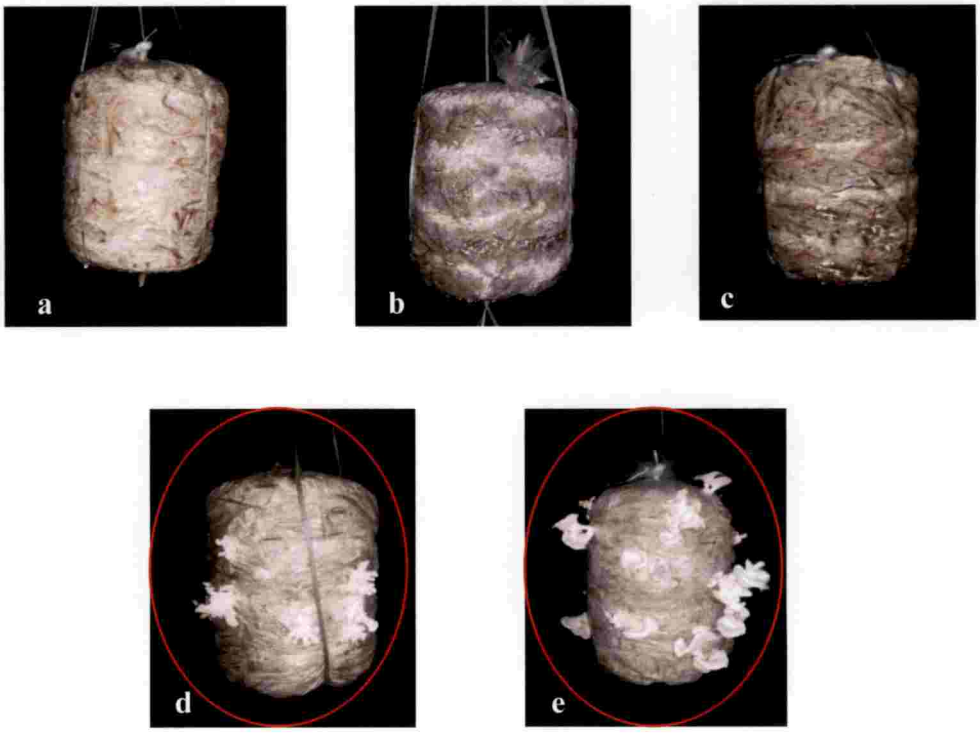


Plate 23. Earliness in primordial initiation in hybrids compared to their parental species of *Pleurotus* on 12th day after spawning; a. *P. djamor*; b. *P. florida*; c. *P. ostreatus*; d. *P. djamor* x *P. florida*; e. *P. djamor* x *P. ostreatus*

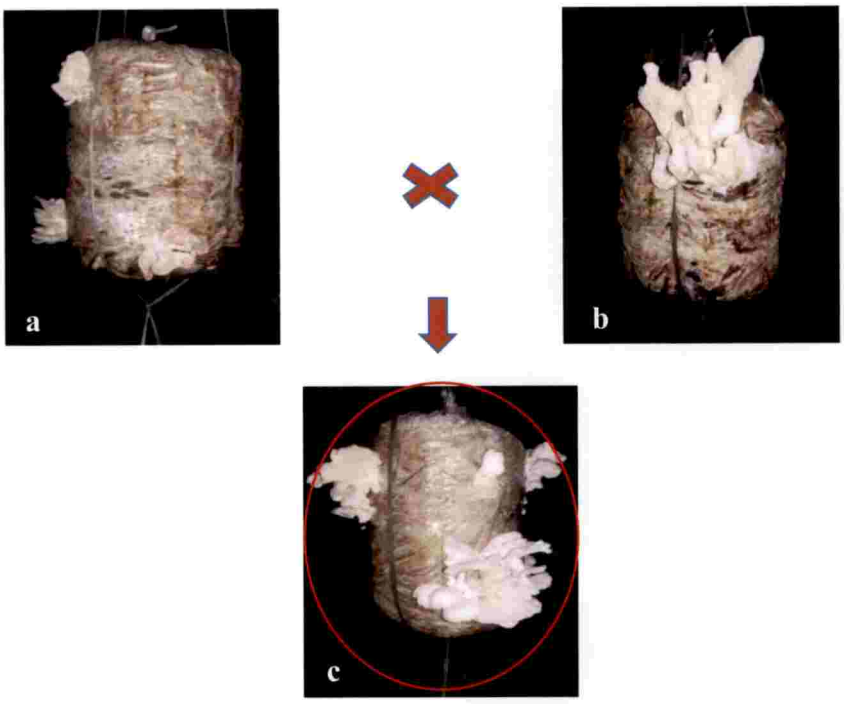


Plate 24. Enhanced yield in hybrid of *P. djamor* and *P. florida* compared to *P. djamor;* a. *P. djamor;* b. *P. florida*; c. *P. djamor* x *P. florida*

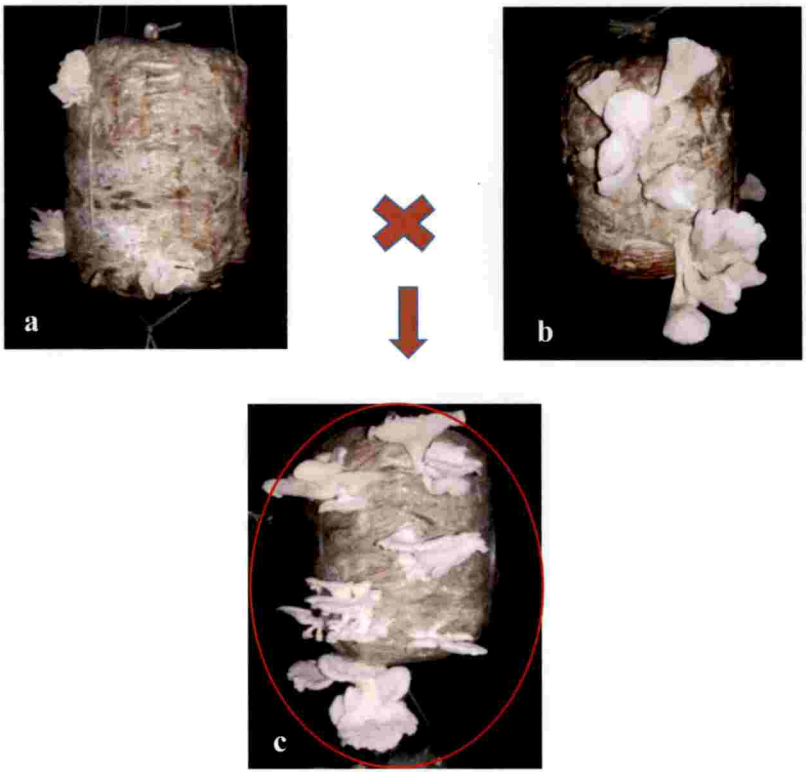


Plate 25. Enhanced yield in hybrid of *P. djamor* and *P. ostreatus* compared to *djamor*; a. P. *djamor;* b. *P. ostreatus*; c. *P. djamor* x *P. ostreatus*

P. ostreatus (43.50 days). Whereas crop period significantly delayed in *P. florida* (53.50 days) and *P. ostreatus* (54.50 days), which did not differ significantly with each other.

Morphological characters of the hybrids were compared to their parents (Table 6, plate 26). Hybrids produced light pink coloured fruiting bodies which were intermediate to pink coloured *P. djamor* and white coloured *P. florida* and *P. ostreatus*. Both *P. florida* and *P. ostreatus* produced delicate sporocarps while, leathery and fibrous sporocarps were produced by *P. djamor*. The hybrids recorded slightly delicate texture, which was an intermediate character to their parents. The pileus dimension of the hybrids was also intermediate to their parents. The pileus length of the hybrid, *P. djamor* x *P. florida* was 5.28 cm which was on par with *P. djamor* (4.80 cm) but significantly smaller than *P. florida* (6.46 cm). Whereas, the pileus breadth of *P. djamor* x *P. florida* measured was 7.35 cm which was significantly superior to *P. djamor* (6.57 cm) but on par with *P. florida* (7.58 cm). In case of the hybrid, *P. djamor* x *P. ostreatus*, the pileus length (6.69 cm) was significantly superior to *P. djamor* but significantly inferior to *P. ostreatus* (7.40 cm). The breadth of the pileus measured was 7.03 cm which was superior to *P. djamor* and significantly inferior to *P. ostreatus* (8.27 cm). The stipe length of hybrids, *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus* was 1.53 cm and 1.64 cm, which were significantly higher than *P. djamor* (0.92 cm) but significantly shorter than *P. florida* (3.33 cm) and *P. ostreatus* (5.58 cm) respectively.

4.5 STUDIES ON STRAIN IMPROVEMENT BY GAMMA IRRADIATION

The cultures of the three selected species of *Pleurotus* were gamma irradiated at doses 20 and 25 Gy from Radio Tracer Laboratory, Kerala Agricultural University, Thrissur. The selected mutants were studied for three generation to ensure stability of the traits. Comparative performances of the parental isolates along with the selected stable mutants were recorded.

Table 6. Biometric observations of sporocarp of species of *Pleurotus* and their hybrids cultivated in paddy straw substrate

Species of *Pleurotus* and their hybrids	Colour	Texture	Pileus length (cm)	Pileus breadth (cm)	Stipe length
*P. djamor**	Pink	Fibrous and leathery	4.80±0.60^{c}	6.57±0.65^{c}	0.92±0.16^{d}
*P. florida**	White	Delicate	6.46±1.11^{b}	7.58±1.45^{b}	3.33±0.44^{b}
*P. ostreatus***	Pale white	Delicate	7.40±1.23^{a}	8.27±0.69^{a}	5.58±0.66^{a}
P. djamor x *P. florida**	Light pink	Slightly delicate	5.28±0.66^{c}	7.35±0.47^{b}	1.53±0.23^{c}
P. djamor x *P. ostreatus**	Light pink	Slightly delicate	6.69±0.37^{b}	7.03±0.42^{bc}	1.64±0.27^{c}
CD(0.05)			0.627	0.603	0.289
SE(m) ±			0.222	0.213	0.102

Values were recorded on 3rd* and 4th day** after primordial initiation

Values are mean ± SD of four replications

Values followed by similar superscripts are not significantly different at 5 % level

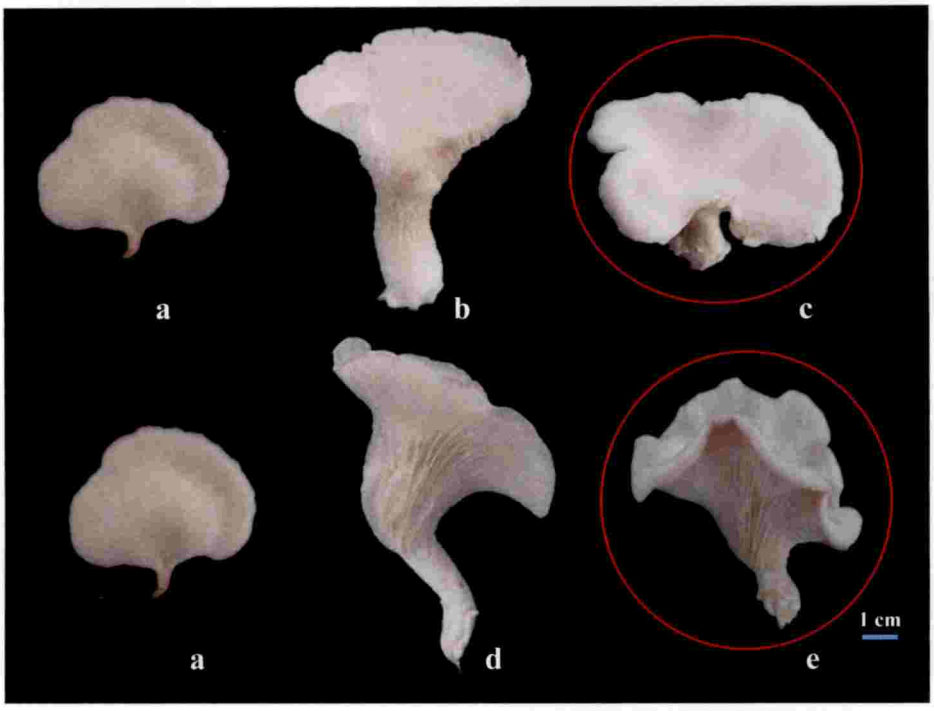


Plate 26. Blended morphological characters of species of *Pleurotus* in their hybrids; a. *P. djamor;* b. *P. florida*; c. *P. djamor* x *P. florida* with increased pileus size and reduced stipe; d. *P. ostreatus*; e. *P. djamor* x *P. ostreatus* with increased pileus size

Comparative performance of *P. djamor* with the mutants at 20 and 25 Gy was analysed (Table 7 and plate 28). Gamma irradiation on *P. djamor* resulted in reduction in number of days needed for complete spawn run in both mutants, *viz.*, *P. djamor* gamma irradiated at 20 and 25 Gy (6.75 and 7.00 days respectively) compared to its parent (9 days). Among the mutants of *P. djamor*, *P. djamor* gamma irradiated at 20 Gy produced pinheads significantly earlier (13 days) than *P. djamor* (14 days) and *P. djamor* gamma irradiated at 25 Gy (14.60), which were statistically on par (Plate 27). Similarly, total crop yield of *P. djamor* irradiated at 20 Gy (428 g kg^{-1}) was significantly superior to *P. djamor* irradiated at 25 Gy (403.25g kg^{-1}) and *P. djamor* (391.75 g kg^{-1}). Whereas sporocarp weight and number of sporocarp in treatments did not show any significant difference with each other.

Influence of gamma irradiation on the performance of *P. florida* was studied and presented in table 8. *P. florida* gamma irradiated at 25 Gy recorded significantly increased sporocarp weight (13.57 g) followed by *P. florida* (12.05 g) and *P. florida* gamma irradiated at 20 Gy (11.75 g) (Plate 29). Significantly higher number of sporocarps were produced by *P. florida* (80) and *P. florida* gamma irradiated at 20 Gy (82.25), which were on par with each other. It was followed by *P. florida* gamma irradiated at 25 Gy which produced an average number of 72 sporocarps. The days taken for pinhead formation was significantly delayed in *P. florida* gamma irradiated at 25 Gy (24.40 days) compared to *P. florida* gamma irradiated at 20 Gy (23 days) and *P. florida* (22 days). Whereas the days required for complete spawn run, total crop yield and total crop period of *P. florida* and its mutants did not vary significantly.

Influence of gamma irradiation on the performance of *P. ostreatus* was studied and presented in table 9. The days taken for complete spawn run significantly reduced in *P. ostreatus* gamma irradiated at 25 Gy (17.40 days). It was followed by *P. ostreatus* (19.80 days) and *P. ostreatus* gamma irradiated at 20 Gy (20.00 days), which were on par with each other. Similarly earliness in primordial initiation was observed in *P. ostreatus* gamma irradiated at 25 Gy

Table 7. Comparative performance of *P. djamor* and their gamma irradiated (20 and 25 Gy) mutants developed through gamma irradiation followed by three generation of selection in paddy straw substrate

Gamma irradiated mutants	Days taken for complete spawn run	Days taken for pinhead formation	Days taken for first harvest	Total yield* (g-fresh weight)	Total crop period (days)	Sporocarp weight (g-fresh weight)	No. of sporocarp	BE (%)
P. djamor	9.00±0.82^{a}	14.00±0.00^{a}	17.00±0.00^{a}	391.75±14.00^{b}	41.25±1.50^{b}	4.95±0.51	95.75±6.80	39.18
P. djamor gamma irradiated at 20 Gy	6.75±0.50^{b}	13.00±0.82^{b}	16.00±0.82^{b}	428.00±30.99^{a}	42.00±0.82^{ab}	4.98±0.27	104.25±9.64	42.80
P. djamor gamma irradiated at 25 Gy	7.00±0.82^{b}	14.60±0.50^{a}	17.60±0.50^{a}	403.25±20.98^{ab}	43.75± 1.89^{a}	4.85±0.40	94.75±4.35	40.32
CD (0.05)	0.881	0.673	0.673	28.850	1.781	N/A	N/A	
SE(m) ±	0.283	0.216	0.216	9.260	0.572	0.158	2.813	

* Total yield from three harvest

Values are mean ± SD of five replications

Values followed by similar superscripts are not significantly different at 5 % level

Table 8. Comparative performance of *P. florida* and their gamma irradiated (20 and 25 Gy) mutants developed through gamma irradiation followed by three generation of selection in paddy straw substrate

Gamma irradiated mutants	Days taken for complete spawn run	Days taken for pinhead formation	Days taken for first harvest	Total yield* (g-fresh weight)	Total crop period (days)	Sporocarp weight (g-fresh weight)	No. of sporocarp	BE (%)
P. florida	19.40±1.29	22.00±0.82^{b}	25.00±0.82^{b}	670.75±58.83	56.50±3.11	12.05±0.87^{b}	80.00±7.30^{a}	67.08
P. florida gamma irradiated at 20 Gy	18.40±0.57	23.00±1.41^{ab}	26.00±1.41^{ab}	650.50±23.40	55.75±4.27	11.75±1.08^{b}	82.25±3.5^{a}	65.05
P. florida gamma irradiated at 25 Gy	19.60±1.50	24.40±1.29^{a}	27.30±1.29^{a}	702.25±14.73	59.00±5.48	13.57±0.37^{a}	72.00±7.74^{b}	70.22
CD (0.05)	N/A	1.461	1.461	N/A	N/A	1.003	7.807	
SE(m) ±	0.476	0.469	0.469	14.536	1.705	0.322	2.506	

* Total yield from three harvest

Values are mean ± SD of five replications

Values followed by similar superscripts are not significantly different at 5 % level

Table 9. Comparative performance of *P. ostreatus* and their gamma irradiated (20 and 25 Gy) mutants developed through gamma irradiation followed by three generation of selection in paddy straw substrate

Gamma irradiated mutants	Days taken for complete spawn run	Days taken for pinhead formation	Days taken for first harvest	Total yield *(g-fresh weight)	Total crop period (days)	Sporocarp weight (g-fresh weight)	No. of sporocarp	BE (%)
P. ostreatus	19.80±0.50[a]	23.00±0.82[a]	27.00±0.82[a]	832.50± 42.77[b]	60.75±2.22[b]	9.43±0.74[a]	101.75±6.24[b]	83.25
P. ostreatus gamma irradiated at 20 Gy	20.00±1.41[a]	23.00±0.82[a]	27.00±0.82[a]	821.75± 27.20[b]	64.50±2.38[a]	9.03±0.35[a]	103.75±9.53[b]	82.18
P. ostreatus gamma irradiated at 25 Gy	17.40±1.26[b]	20.00±1.82[b]	24.00±1.82[b]	939.80± 19.01[a]	53.75±1.89[c]	8.10±0.59[b]	124.00±15.12[a]	93.98
CD (0.05)	0.985	1.505	1.531	37.715	2.631	0.706	13.196	
SE(m) ±	0.316	0.483	0.525	12.106	0.845	0.227	4.236	

* Total yield from three harvest

Values are mean ± SD of five replications

Values followed by similar superscripts are not significantly different at 5 % level

Table 10. Comparative performance of species of *Pleurotus* and their gamma irradiated (20 and 25 Gy) mutants developed through gamma irradiation followed by three generation of selection

Gamma irradiated mutants	Days taken for complete spawn run	Days taken for pinhead formation	Days taken for first harvest	Total yield* (g-fresh weight)	Total crop period(days)	Sporocarp weight (g-fresh weight)	No. of sporocarp	BE (%)
P. djamor	9.00±0.82^{d}	14.00±0.00^{de}	17.00±0.00^{de}	391.75±14.00^{e}	41.25±1.50^{e}	4.95±0.51^{e}	95.75±6.80^{b}	39.18
P. florida	19.40±1.29^{ab}	22.00±0.82^{b}	25.00±0.82^{bc}	670.75±58.83^{cd}	56.50±3.11^{bcd}	12.05±0.87^{b}	80.00±7.30^{c}	67.08
P. ostreatus	19.80±0.96^{a}	23.00±0.82^{ab}	27.00±0.82^{a}	832.50±42.77^{b}	60.75±2.22^{ab}	9.43±0.74^{c}	101.75±6.24^{b}	83.25
P. djamor (20 Gy)	6.75±0.50^{e}	13.00±0.82^{e}	16.00±0.82^{e}	428.00±30.99^{e}	42.00±0.82^{e}	4.98±0.27^{e}	104.25±9.64^{b}	42.80
P. djamor (25 Gy)	7.00±0.82^{e}	14.60±0.50^{d}	17.60±0.50^{d}	403.25±20.98^{e}	43.75±1.89^{e}	4.85±0.40^{e}	94.75±4.35^{b}	40.32
P. florida (20 Gy)	18.40±0.57^{bc}	23.00±1.41^{ab}	26.00±1.41^{ab}	650.50±23.40^{d}	55.75±4.27^{cd}	11.75±1.08^{b}	82.25±3.5^{c}	65.05
P. florida (25 Gy)	19.60±1.50^{ab}	24.40±1.29^{a}	27.30±1.29^{a}	702.25±14.73^{c}	59.00±5.48^{bc}	13.57±0.37^{a}	72.00±7.74^{c}	70.22
P. ostreatus (20Gy)	20.00±0.82^{a}	23.00±0.82^{ab}	27.00±0.82^{a}	821.75±27.20^{b}	64.50±2.38^{a}	9.03±0.35^{c}	103.75±9.53^{b}	82.18
P. ostreatus (25Gy)	17.40±0.58^{c}	20.00±1.82^{c}	24.00±1.82^{c}	939.80±19.01^{a}	53.75±1.89^{d}	8.10 ± 0.59^{d}	124.00±15.12^{a}	93.98
CD (0.05)	1.354	1.520	1.520	45.489	4.31	0.922	12.321	
SE(m) ±	0.464	0.525	0.525	15.593	1.477	0.316	4.224	

* Total yield from three harvest

Values are mean ± SD of five replications

Values followed by similar superscripts are not significantly different at 5 % level

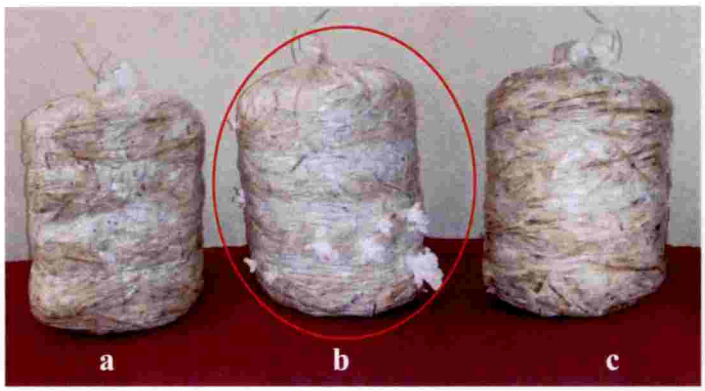


Plate 27. Earliness in primordial initiation in *P. djamor* gamma irradiated at 20 Gy on 13th day after spawning compared to *P. djamor* and *P. djamor* gamma irradiated at 25 Gy; a. *P. djamor;* b. Gamma mutant at 20 Gy; c. Gamma mutant at 25 Gy

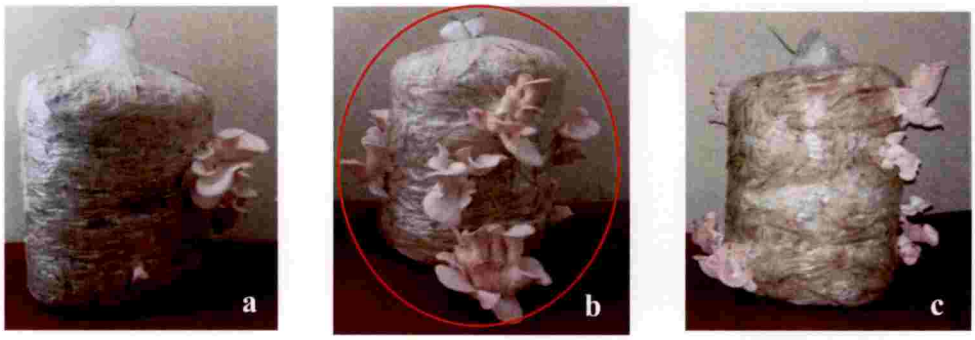


Plate 28. Enhanced yield in *P. djamor* gamma irradiated at 20 Gy compared to *P. djamor* and *P. djamor* gamma irradiated at 25 Gy; a. *P. djamor;* b. Gamma mutant at 20 Gy; c. Gamma mutant at 25 Gy

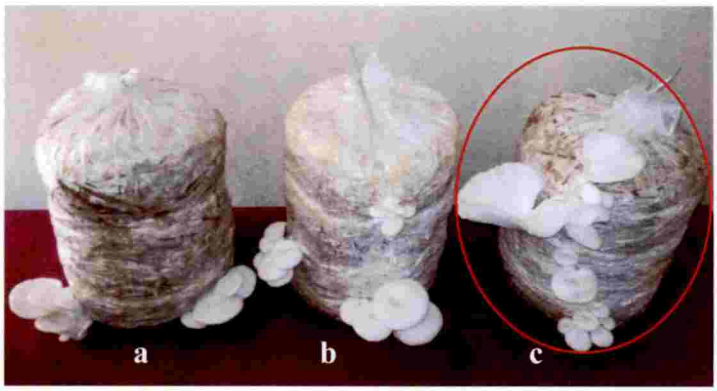


Plate 29. Larger sporocarps of *P. florida* gamma irradiated at 25 Gy compared to *P. florida* and *P. florida* gamma irradiated at 20 Gy; a. *P. florida;* b. Gamma mutant at 20 Gy; c. Gamma mutant at 25 Gy

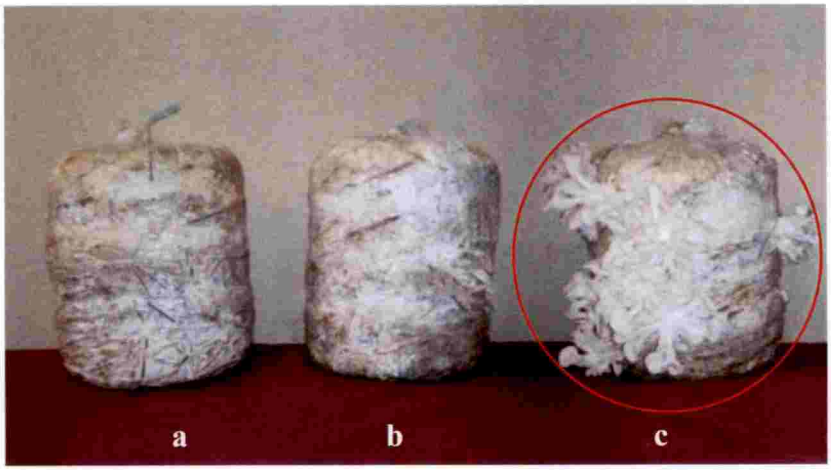


Plate 30. Earliness in primordial initiation in *P. ostreatus* gamma irradiated at 25 Gy on 20th day after spawning compared to *P. ostreatus* and *P. ostreatus* gamma irradiated at 20 Gy; a. *P. ostreatus*; b. Gamma mutant at 20 Gy; c. Gamma mutant at 25 Gy

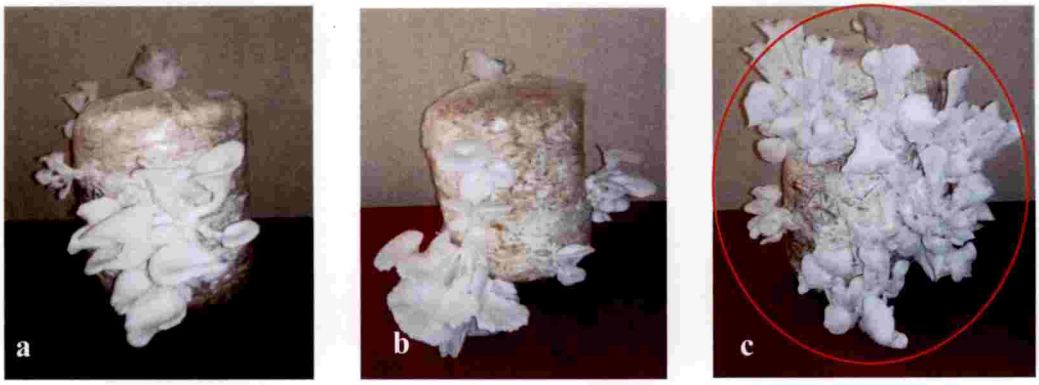


Plate 31. Enhanced yield in *P. ostreatus* gamma irradiated at 25 Gy compared to *P. ostreatus* and *P. ostreatus* gamma irradiated at 20 Gy; a. *P. ostreatus*; b. Gamma mutant at 20 Gy; c. Gamma mutant at 25 Gy

(20 days) followed by *P. ostreatus* and *P. ostreatus* irradiated at 20 Gy, which were on par with each other (Plate 30). *P. ostreatus* gamma irradiated at 25 Gy recorded increased yield with reduced crop period. It produced significantly higher yield of 939.80 g kg^{-1} compared to its parent (832.50 g kg^{-1}) and mutant at 20 Gy (821.75 g kg^{-1}), which were statistically on par with each other (Plate 31). Also it showed reduced crop period (53.75 days) followed by *P. ostreatus* (60.75 days) and *P. ostreatus* gamma irradiated at 20 Gy (64.50 days). Sporocarp weight of *P. ostreatus* (9.43 g) and *P. ostreatus* gamma irradiated at 20 Gy (9.03 g) were on par with each other. It was followed by *P. ostreatus* gamma irradiated at 25 Gy recording reduced sporocarp weight of 8.10 g. Maximum number of sporocarps (124) were produced by *P. ostreatus* gamma irradiated at 25 Gy. It was followed by *P. ostreatus* gamma irradiated at 20 Gy (103.75) and *P. ostreatus* (101.75), which were statistically on par with each other.

Comparative performance of *P. djamor*, *P. florida* and *P. ostreatus*, and their mutants was analysed in table 10. *P. ostreatus* gamma irradiated at 25 Gy recorded maximum yield compared to other species of *Pleurotus* and their mutants. Whereas, gamma irradiation of *P. djamor* at 20 Gy resulted in early emergence of pinheads (13^{th} day after spawning) compared to all other species of *Pleurotus* and their mutants.

4.6 COMPARISON OF THE BEST CULTURES

4.6.1 Yield

The native isolates and the best cultures from the above experiments, *P. djamor* x *P. florida*, *P. djamor* x *P. ostreatus*, *P. djamor* gamma irradiated at 20 Gy, *P. ostreatus* gamma irradiated at 25 Gy were selected for their comparative performance (Table 11, plate 32). The BE of all the treatments were compared and found that *P. ostreatus* gamma irradiated at 25 Gy recorded 91.72 per cent BE, which was 12.3 % higher over *P. ostreatus* (81.65 %). Whereas, hybrids were found to be superior to their parents with reference to increased BE

Table 11. Comparative performance of species of *Pleurotus* and their improved strains developed through hybridisation and gamma irradiation followed by three generation of selection

Species of *Pleurotus* and their improved strains	Days taken for complete spawn run	Days taken for pinhead formation	Days taken for first harvest	Total yield* (g-fresh weight)	Total crop period (days)	Sporocarp weight (g-fresh weight)	No. of sporocarp	BE (%)
P. djamor	9.75±0.96^{c}	15.00±0.82^{c}	18.00±0.82^{c}	399.00±11.40^{f}	40.75±1.71^{d}	5.02±0.57^{d}	90.75±4.11^{c}	39.90
P. florida	20.25±0.96^{a}	24.75±0.96^{a}	27.75±0.96^{a}	671.75±53.74^{c}	54.50±1.91^{b}	12.1±0.84^{a}	78.00±5.48^{d}	67.18
P. ostreatus	20.50±0.58^{a}	24.00±0.82^{a}	28.00±0.82^{a}	816.50±35.46^{b}	59.75±2.87^{a}	9.5±0.74^{b}	103.00±7.02^{b}	81.65
P. djamor x *P. florida*	7.75±0.5^{d}	13.00±0.82^{d}	16.00±0.82^{d}	540.50±36.53^{e}	44.00±0.82^{c}	8.10±0.57^{c}	90.50±3.11^{c}	54.05
P. djamor x *P. ostreatus*	7.50±0.58^{d}	12.25±0.50^{d}	15.25±0.58^{d}	597.50±20.82^{d}	44.75±1.23^{c}	8.05±0.31^{c}	100.25±3.20^{bc}	59.75
P. djamor gamma irradiated at 20 Gy	7.25±0.50^{d}	13.50±0.58^{d}	16.50±0.58^{d}	413.75±15.58^{f}	42.25±0.82^{cd}	5.02±0.43^{d}	101.25±8.10^{bc}	41.38
P. ostreatus gamma irradiated at 25 Gy	17.75±0.50^{b}	20.75±1.26^{b}	25.50±1.73^{b}	917.25±15.13^{a}	53.50±1.89^{b}	8.18±0.51^{c}	122.25±16.66^{a}	91.72
CD (0.05)	1.009	1.272	1.272	45.17	2.835	0.876	11.992	
SE(m) ±	0.341	0.430	0.430	15.25	0.957	0.296	4.050	

* Total yield from three harvest

Values are mean ± SD of four replications

Values followed by similar superscripts are not significantly different at 5 % level

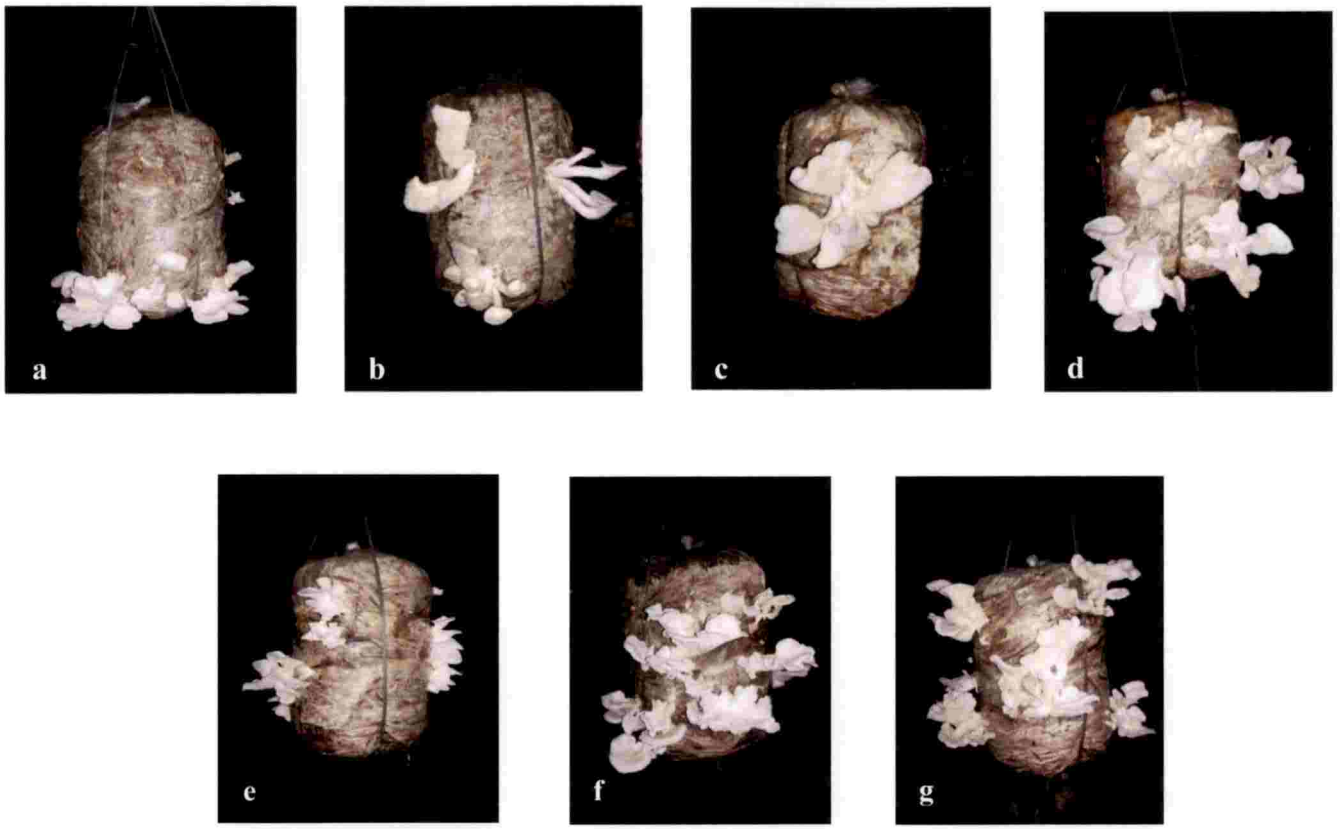


Plate 32. Enhanced yield in selected strains of species of *Pleurotus* developed through hybridisation and gamma irradiation followed by three generation of studies compared to parents. a. *P. djamor;* b. *P. florida*; c. *P. ostreatus*; d. *P. ostreatus* gamma irradiated at 25Gy; e. *P. djamor* gamma irradiated at 20 Gy; f. *P. djamor* x *P. florida*; g. *P. djamor* x *P. ostreatus*

with reduced crop period. Increased BE over *P. djamor* was recorded for *P. djamor* x *P. ostreatus* (49.74 %), followed by *P. djamor* x *P. florida* (35.46 %). The hybrids, *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus* recorded reduced crop period by 10.5 days and 15 days compared to *P. florida* and *P. ostreatus* respectively.

4.6.2 Proximate Constituents of species of *Pleurotus*

Proximate constituents of the three *Pleurotus* spp. along with the hybrids, *P. djamor* x *P. florida*, *P. djamor* x *P. ostreatus* and the mutant of *P. ostreatus* gamma irradiated at 25 Gy were evaluated. The results on moisture content, carbohydrate, protein and fibre are presented in Table 12.

4.6.2.1 Estimation of Moisture Content

Moisture content of *Pleurotus* spp. was determined based on fresh weight. *P. florida* recorded the maximum moisture content (92.16 %) followed by *P. ostreatus* (89.14 %), which was significantly different from each other. *P. djamor* x *P. florida*, *P. djamor* x *P. ostreatus* and *P. ostreatus* gamma irradiated at 25 Gy contained 87.81, 88.31 and 87.71 per cent moisture content which were statistically on par. Minimum moisture content was observed in *P. djamor* (85.97 %) which differed significantly from other *Pleurotus* spp.

4.6.2.2 Estimation of Carbohydrate Content

P. ostreatus gamma irradiated at 25 Gy, *P. ostreatus* and *P. florida* recorded highest carbohydrate content of 56.33, 55.77 and 52.42 per cent. This was followed by hybrid *P. djamor* x *P. florida* (50.44 %) and *P. djamor* x *P. ostreatus* (51.68 %), which were statistically on par. Minimum carbohydrate content (45.79 %) was recorded in *P. djamor*.

Table 12. Analysis of proximate contents in species of *Pleurotus* and their improved strains developed through hybridisation and gamma irradiation followed by three generation of selection in paddy straw substrate

Species of *Pleurotus* and their improved strains	Moisture (%)*	Carbohydrate (%)**	Protein (%)**	Fibre (%)**
P. djamor	85.97±1.29^{c}	45.79±1.66^{d}	23.33±1.04^{bc}	9.73±0.68^{cd}
P. florida	92.16±1.12^{a}	52.42±1.32^{bc}	21.61±1.21^{c}	8.38±0.52^{d}
P. ostreatus	89.14±1.05^{b}	55.77±0.77^{ab}	22.31±1.06^{c}	11.54±1.38^{b}
P. djamor x *P. florida*	87.81±1.17^{bc}	50.44±1.94^{c}	26.54±1.05^{a}	14.07±1.07^{a}
P. djamor x *P. ostreatus*	88.31±1.18^{bc}	51.68±1.60^{c}	25.14±1.24^{ab}	13.35±0.59^{a}
P. ostreatus gamma irradiated at 25 Gy	87.71±1.06^{bc}	56.33±1.36^{a}	21.47±0.86^{c}	10.92±0.82^{bc}
CD (0.05)	2.948	3.765	1.939	1.590
SE(m) ±	0.956	1.220	0.629	0.516

*Fresh weight basis; ** Dry weight basis

Values are mean ± SD of four replications

Values followed by similar superscripts are not significantly different at 5 % level

4.6.2.3 Estimation of Protein Content

Protein content was recorded significantly high in *P. djamor* x *P. florida* (26.54 %) followed by *P. djamor* x *P. ostreatus* (25.14 %) and *P. djamor* (23.33 %). *P. florida*, *P. ostreatus* and *P. ostreatus* gamma irradiated at 25 Gy contained 21.61, 22.31 and 21.47 per cent protein content, which did not vary significantly.

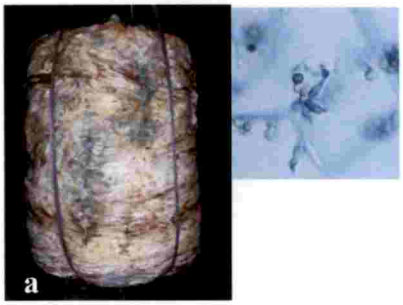
4.6.2.4 Estimation of Crude Fibre

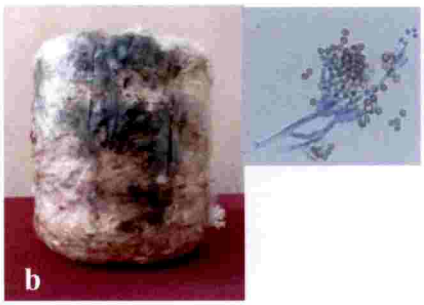
Maximum crude fibre content was estimated in hybrid of *P. djamor* x *P. florida* (14.07 %) and *P. djamor* x *P. ostreatus* (13.35 %), which were not significantly different. This was followed by *P. ostreatus*, *P. ostreatus* gamma irradiated at 25 Gy and *P. djamor*, which had 11.54, 10.92 and 9.73 per cent fibre content. Whereas least crude fibre content was recorded in *P. florida* (8.38 %).

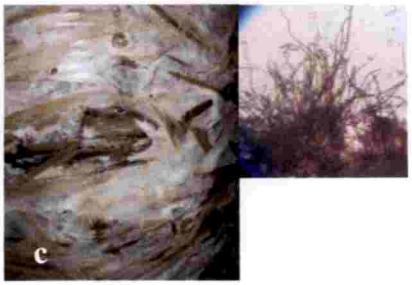
4.7 PEST AND DISEASE INCIDENCE

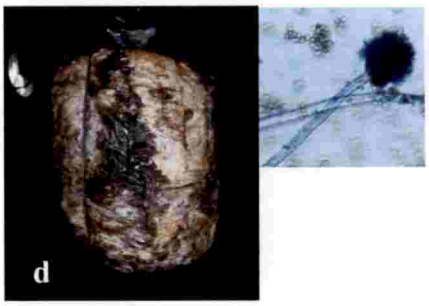
The major pests observed in mushroom beds were springtail, phorid flies and staphilinid beetle (Plate 34). Most predominant pest noted during cultivation trial was springtails (*Seira* sp.). During the initial stage, the infestation resulted in reduced spawn run, thus affected the flush development. Springtails were mainly seen crowded at the lower portion of the pileus and on the stipe. It showed characteristic jumping movement and remained in between the gills. The grubs of staphilinid beetles were found to make tunnels in the stipe and lower portion of the pileus. Ultimately, it resulted in decaying of the fruiting body. The larvae of phorid flies were found to feed the mushroom mycelium in the early stages. During the cropping period, larvae made tunnels in the mushroom and resulted in decay of the fruiting bodies. Adults were seen on the sporocarp.

The fungal contaminants observed in mushroom beds were *Coprinus* sp., *Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp., and *Chaetomium* sp. (Plate 33). Among the contaminants *Trichoderma* spp. caused the significant losses. A white









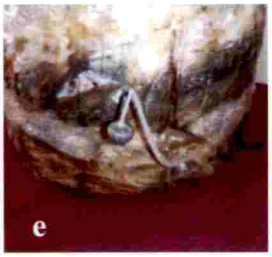


Plate 33. Fungal contaminants observed in mushroom beds of species of *Pleurotus*; a. *Trichoderma* sp.; b. *Penicillium* sp.; c. *Chaetomium* sp.; d. *Aspergillus* sp.; e. *Coprinus* sp.

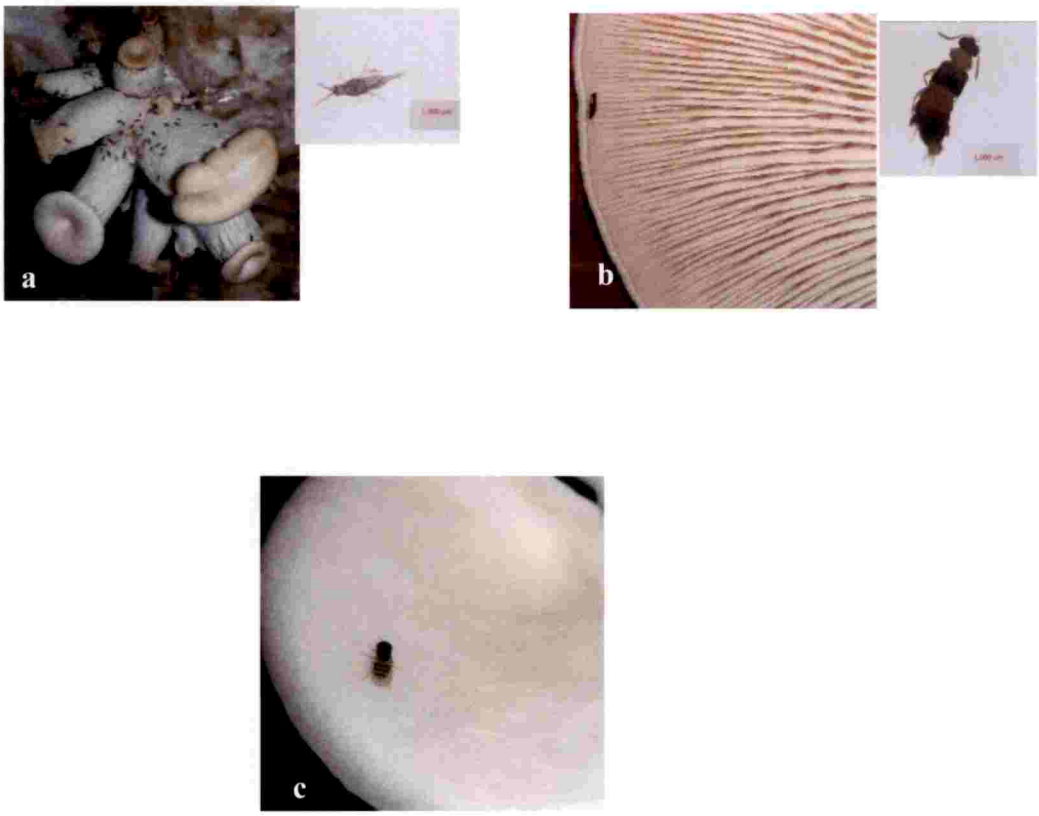


Plate 34. Pests observed in mushroom beds of species of *Pleurotus*; a. *Seira* sp.; b. Staphylinid beetle; c. *Megaselia* sp.

coloured mycelium resembling that of mushroom was produced by *Trichoderma* spp. on mushroom beds. Later the mycelial mat was turned to green colour due to excessive sporulation. At the earlier stage, the infection spread faster than the mushroom mycelium, resulting in poor spawn run and reduced yield. *Aspergillus* sp. and *Penicillium* sp. also resulted in reduced yield.

Chaetomium sp. seriously affected the mycelial run in mushroom beds and the mycelium was found to occur in white patches. Initially the fungus consisted of greyish white mycelium, later producing perithecia. The perithecia were opaque, superficial and globose with tuft of dark bristles of setae.

During the harvesting of mushroom, *Coprinus* sp. was found to compete with the *Pleurotus* spp. These were slender bell shaped mushrooms, creamish coloured at first and later changing to bluish black with scales on the surface. A blackish slimy mass was then produced by *Coprinus* sp. due to its autodigestion.

4.8 RAPD ANALYSIS

4.8.1 DNA Isolation

DNA of the parents *P. djamor*, *P. florida*, *P. ostreatus* and their improved strains *P. djamor* x *P. florida*, *P. djamor* x *P. ostreatus*, *P. djamor* gamma irradiated at 20 Gy, *P. florida* and *P. ostreatus* gamma irradiated at 25 Gy were isolated using DNeasy plant mini kit (Qiagens).

4.8.2 PCR Amplification

The isolated DNA of parents with their hybrids were amplified using RAPD primer OPT 5 (GGGTTTGGCA) and parents with their mutants were amplified using OPS 5 (TTTGGGGCCT).

Out of total amplicons produced using the primer OPT 5, three amplicons of size 980 bp, 938 bp and 500 bp were monomorphic between parent, *P. florida*

and *P. djamor* x *P. florida*, while four amplicons of size 957 bp, 938 bp, 907 bp and 500 bp were monomorphic between parent, *P. djamor* and *P. djamor* x *P. florida*. All the other bands were polymorphic. Four amplicons of size 938 bp, 907 bp, 738 bp and 500 bp were monomorphic between *P. djamor* and *P. djamor* x *P. ostreatus*, whereas two amplicons of size 980 bp and 500 bp were monomorphic between *P. ostreatus* and *P. djamor* x *P. ostreatus* (Plate 35).

Primer OPS 5 was used for amplifying the parents and their mutants *viz.*, *P. djamor* gamma irradiated at 20 Gy, *P. florida* gamma irradiated at 25 Gy and *P. ostreatus* gamma irradiated at 25 Gy. Two amplicons of size 600 bp and 910 bp were polymorphic between *P. florida* and its mutant, while amplicons of size 550 bp and 600 bp were polymorphic between *P. ostreatus* and its mutant. One polymorphic amplicon of size 850 bp was present between *P. djamor* and its mutant (Plate 36).

Based on the presence or absence of amplicons, bands were scored (Table 13). Statistical analysis was done for the results obtained with the primers. The bivariate data thus obtained were then analysed to generate Jaccard's similarity coefficient (Table 14). The pair wise coefficient values ranged from 0.143 to 0.750. The hybrid, *P. djamor* x *P. florida* had 66.7 per cent and 50 per cent similarity with *P. djamor* and *P. florida* respectively. While *P. djamor* x *P. ostreatus* had 57.1 per cent similarity with *P. djamor* and 28.6 per cent similarity with *P. ostreatus*. The matrix was subjected to UPGMA to develop dendrogram. The hybrids formed a single cluster with the *P. djamor*. Among the hybrids, *P. djamor* x *P. florida* recorded more similarity to *P. djamor*.

The bivariate data obtained from RAPD profile of *Pleurotus* spp. with mutants was analysed (Table 15). Using Jaccard's similarity coefficient (Table 16), it was recorded that the better mutant *P. ostreatus* gamma irradiated at 25 Gy exhibited 22 per cent polymorphism with the parent. While, *P. djamor* and *P. florida* had 16.70 and 25 per cent polymorphism with their respective parents.

Table 13. RAPD scoring pattern of species of *Pleurotus* and their hybrids

Amplicon size	*P. florida*	*P. djamor*	*P. ostreatus*	*P. djamor* x *P. florida*	*P. djamor* x *P. ostreatus*
980 bp	1	0	1	1	1
957 bp	0	1	0	1	0
938 bp	1	1	0	1	1
907 bp	0	1	0	1	1
738 bp	0	1	0	0	1
500 bp	1	1	1	1	1
397 bp	0	0	0	0	1
271 bp	1	0	1	0	0

Table 14. Similarity matrix for Jaccard's coefficient of species of *Pleurotus* and their hybrids

	P. djamor	*P. florida*	*P. ostreatus*	*P. djamor* x *P. florida*	*P. djamor* x *P. ostreatus*
P. djamor	1	0.286	0.143	0.667	0.571
P. florida		1	0.750	0.500	0.429
P. ostreatus			1	0.333	0.286
P. djamor x *P. florida*				1	0.571
P. djamor x *P. ostreatus*					1

Table 15. RAPD scoring pattern of species of *Pleurotus* and their gamma irradiated mutants

Amplicon size	*P. florida*	*P. florida* mutant 25 Gy	*P. ostreatus*	*P. ostreatus* mutant 25 Gy	*P. djamor*	*P. djamor* mutant 20 Gy
350 bp	1	1	1	1	0	0
500 bp	1	1	1	1	1	1
550 bp	0	0	0	1	0	0
600 bp	0	1	0	1	0	0
700 bp	1	1	1	1	1	1
850 bp	0	0	0	0	1	0
910 bp	0	1	1	1	1	1
950 bp	1	1	1	1	1	1
980 bp	1	1	1	1	0	0
1200 bp	1	1	1	1	1	1

Table 16. Distance matrix for Jaccard's coefficient of species of *Pleurotus* and their gamma irradiated mutants

	P. florida	*P. florida* mutant (25 Gy)	*P. ostreatus*	*P. ostreatus* mutant (25 Gy)	*P. djamor*	*P. djamor* mutant (20 Gy)
P. florida	0	0.250	0.143	0.333	0.500	0.429
P. florida mutant (25 Gy)		0	0.125	0.111	0.444	0.375
P. ostreatus			0	0.220	0.375	0.286
P. ostreatus mutant (25 Gy)				0	0.500	0.444
P. djamor					0	0.167
P. djamor mutant (20 Gy)						0

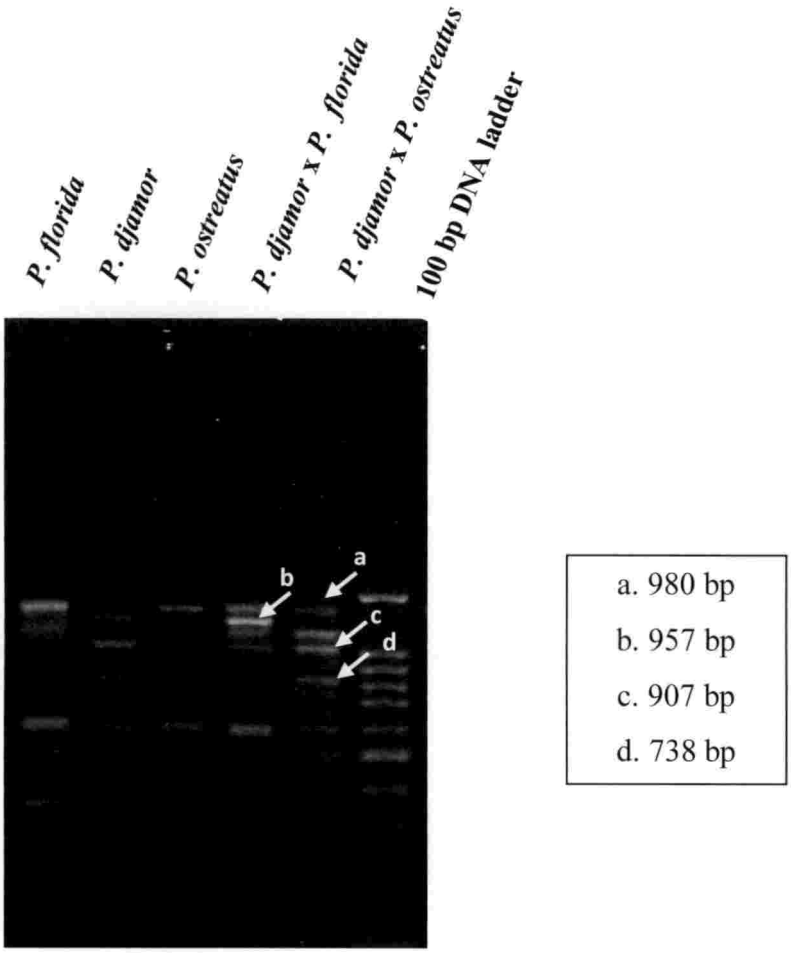
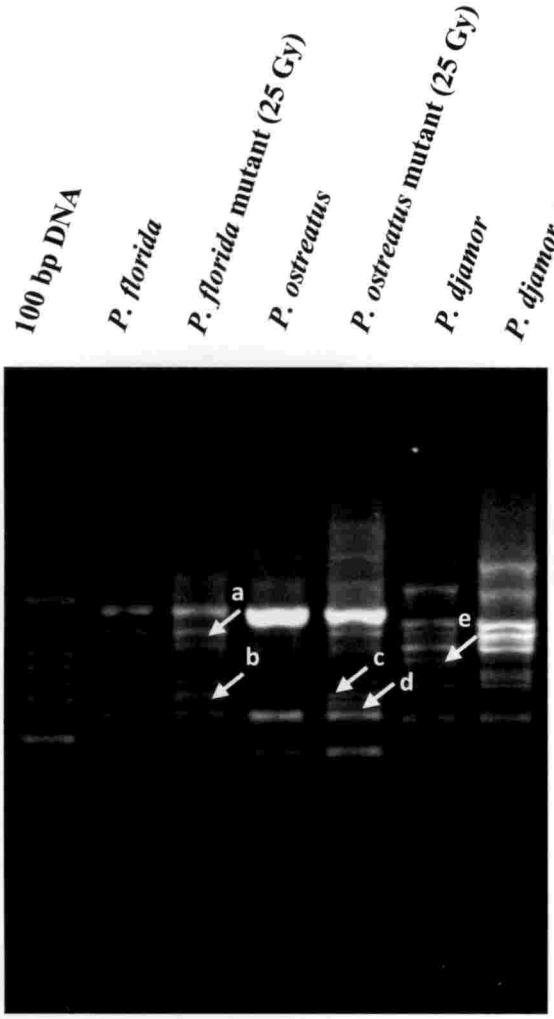


Plate 35. RAPD profile of species of *Pleurotus* and their hybrids amplified by primer OPT 5 (GGGTTTGGCA); *P. djamor* x *P. florida* share 980 bp of *P. florida* and 957 bp and 907 bp of *P. djamor*; *P. djamor* x *P. ostreatus* share 938 bp, 907 bp and 738 bp of *P. djamor* and 980 bp of *P. ostreatus*



a. 910 bp
b. 600 bp
c. 600 bp
d. 550 bp
e. 850 bp

Plate 36. RAPD profile of species of *Pleurotus* and their gamma irradiated mutants amplified by primer OPS 5 (TTTGGGGCCT); *P. florida* and *P. florida* gamma irradiated at 25 Gy are polymorphic at amplicons of size 600 bp and 910 bp; *P. ostreatus* and *P. ostreatus* gamma irradiated at 25 Gy are polymorphic at 550 bp and 600 bp; *P. djamor* and *P. djamor* gamma irradiated at 20 Gy are polymorphic at 850 bp

Discussion

5. DISCUSSION

Oyster mushroom (*Pleurotus* spp.) commonly called as Dhingri in India, stands second among world production. Due to awareness on neutraceutical and therapeutic properties, the demand for mushroom is increasing day by day. But the cultivated mushrooms face the problems of loss of genetic diversity and strain degeneration (Wang *et al.,* 2012). Also the existing cultivated mushrooms with high yield have longer duration, whereas short duration mushrooms yield low. Thus development of improved strain from the existing germplasm is inevitable for enhanced production in short period.

P. djamor and *P. florida* are the highly appreciated mushrooms in Kerala. *P. djamor* produced pink coloured fruiting bodies of small clusters within reduced cropping period. However, its yield efficiency is very low. Moreover, the leathery nature of sporocarp makes it less preferred for consumption. *P. florida* and *P. ostreatus* have high BE but have comparatively longer duration. The delicate texture of the fruiting body makes it inconvenient for packing and transit. Therefore, the present study emphasizes in the development of improved strain of *Pleurotus* of fleshy pileus with cluster type growing pattern and better BE within short cropping period.

5.1 MORPHOLOGICAL CHARACTERISATION OF SPOROCARP OF NATIVE ISOLATES

5.1.1 Macroscopical Observations

The sporocarps from the native isolates *viz.*, *P. djamor, P. florida* and *P. ostreatus* were studied for their morphological characters. The pileus colour, pileus dimension, stipe length, sporocarp weight and texture of the species of *Pleurotus* were recorded.

P. djamor produced pink coloured leathery sporocarps with smaller pileus. It was characterized by spathulate to flabelliform pileus with entire margin.

Whereas *P. florida* and *P. ostreatus* produced delicate sporocarps with comparatively larger pileus dimension and increased stipe length. *P. florida* yielded white coloured fleshy sporocarps with entire, enrolled type pileus margin. Greyish white coloured sporocarps with entire margin were produced by *P. ostreatus*. The average fresh sporocarp weight of the three species of *Pleurotus* was recorded. It was found that the fresh sporocarp weight of *P. djamor* was significantly lower compared to other two species of *Pleurotus* studied.

The pileus dimension of *P. florida* measured was 6.46 cm × 7.58 cm which is larger than that of *P. djamor* which recorded 4.80 cm × 6.57 cm (Shukla and Jaitly, 2011; Krishnapriya *et al.,* 2017). The gills were attached decurrently to the stipe in both *P. djamor* and *P. florida*. Whereas in *P. ostreatus*, the gills were arranged falcate decurrently to its lengthy stipe. Among the isolates, the gills were arranged more crowded in *P. djamor* compared to *P. florida* and *P. ostreatus*.

P. djamor and *P. florida* attained harvesting maturity in three days from pinhead emergence (Jose, 2018). Whereas *P. ostreatus* recorded an average of four days from pinhead formation to complete maturity. Compared to *P. djamor*, both *P. florida* and *P. ostreatus* produced pinheads in bunches.

5.1.2 Microscopical Observations

Microscopical characteristics of hyphae, spores and cystidia of the three *Pleurotus* were studied. Hyphae of all three *Pleurotus* spp. were septate, branched and hyaline with clamp connections. The width of the hyphae did not vary significantly among each other. The three isolates had clavate shaped basidia with four basidiospores produced on its sterigmata. The dimensions of basidia were more or less similar in the *Pleurotus* spp. with slightly broader basidia for *P. ostreatus*. Basidiospores of the isolates were hyaline and cylindrical in shape, produced on tetrasterigmatic clavate shaped basidia (Junior *et al.,* 2010; Biswas *et al.,* 2011; Das *et al.,* 2015; Acharya *et al.*, 2017). *P. djamor* and *P. florida* produced basidiospores of equal dimension whereas comparatively

smaller spores were produced by *P. ostreatus*. Subventricose to clavate shaped cheilocystidia were observed in all the *Pleurotus* spp. studied and were present between clusters of basidia. Compared to *P. florida* and *P. ostreatus* small sized cheilocystidia were produced by *P. djamor*.

Spore print of the *P. djamor* was light pink in colour which later changed to creamish white. White spore print was produced by other two *Pleurotus* spp. *viz. P. florida* and *P. ostreatus* (Jose, 2018).

5.2 RE-ISOLATION AND PURE CULTURING OF SELECTED ISOLATES AND STUDYING MORPHOLOGICAL CHARACTERS OF THE CULTURE

Partially matured healthy, pest and disease free sporocarps of the three *Pleurotus* spp. were tissue cultured in PDPA medium. Krishnapriya (2018) identified PDPA as the best medium compared to PDA, MEA, OMA and CEA, for the three isolates of *Pleurotus* spp. *viz. P. cystidiosus coremial, P. cystidiosus* non-coremial and *P. opuntia.*

The culture of *P. djamor* was cream coloured thick cottony mycelium with concentric pattern on PDPA media, whereas cultures of *P. florida* exhibited white fluffy growth with even margin (Krishnapriya *et al.,* 2017). Thick stranded, greyish white coloured mycelium with radiating margin was observed in *P. ostreatus*. *P. florida* recorded faster mycelial growth and the days to complete the growth in 9 cm (diameter) petri plate was minimum (nine days). It was followed by *P. ostreatus* (10 days) and *P. djamor* (12 days).

The mycelial growth rate was significantly higher for *P. florida* compared to *P. djamor* (Jose, 2018). Variation in growth rate may be due to genetic differences found in different species of genus *Pleurotus* (Sardar *et al.,* 2015) or specificity of *Pleurotus* mycelium to different culture media (Singh and Singh, 2018).

5.3 STUDIES ON STRAIN IMPROVEMENT BY HYBRIDISATION THROUGH SPAWN MIXING

Mixing spawn of two different strains found to be the easiest way of developing hybrids. Anitha (1998) reported development of new strain by hybridisation through spawn mixing. The recombined culture of *P. sajor-caju* x *P. florida* produced creamish sporocarp whereas the parents had grey and white sporocarps. The yield was also superior to one of the parent *P. florida* and on par with *P. sajor-caju*. Based on the above report, a similar attempt was made for the three *Pleurotus* spp. studied. Spawn mixed beds, *viz., P. djamor* + *P. florida* and *P. djamor* + *P. ostreatus* completed its spawn run significantly earlier than the parents *P. florida* and *P. ostreatus*. But the days required for complete spawn run and pinhead initiation were minimum for *P. djamor* (Figure 4).

P. djamor completed its spawn run earlier than the other two isolates. Because of the difference in spawn run rate of their parent isolates which were mixed together, sporocarps of both parents emerged separately on the spawn mixed beds. Thus the sporocarp of *P. djamor* emerged earlier in the spawn mixed beds of *P. djamor* + *P. florida* and *P. djamor* + *P. ostreatus*. Total crop yield of spawn mixed beds was significantly low compared to the parents (Figure 5). BE was only 25.83 and 23.55 per cent respectively for the spawn mixed beds of *P. djamor* + *P. florida* and *P. djamor* + *P. ostreatus*. Also the beds prepared out of mixed spawn completed its crop period significantly earlier and started decaying *i.e.,* the crop period was 30.75 and 33.24 days for the spawn mixed beds of *P. djamor* + *P. florida* and *P. djamor* + *P. ostreatus* respectively. The mycelium of *P. djamor* have better ability to colonize vast agricultural lignocellulosic wastes ensuing fast completion of the production cycle, thus accelerate the time to fructify (Yang *et al.*, 2013).

Based on the above observations, it is clear that hybridisation or crossing did not take place by spawn mixing and thus did not have any significance in strain improvement of the *Pleurotus* spp. used in the study. Fewer chance of crossing mycelia through spawn mixing may be due to low probability of nuclear

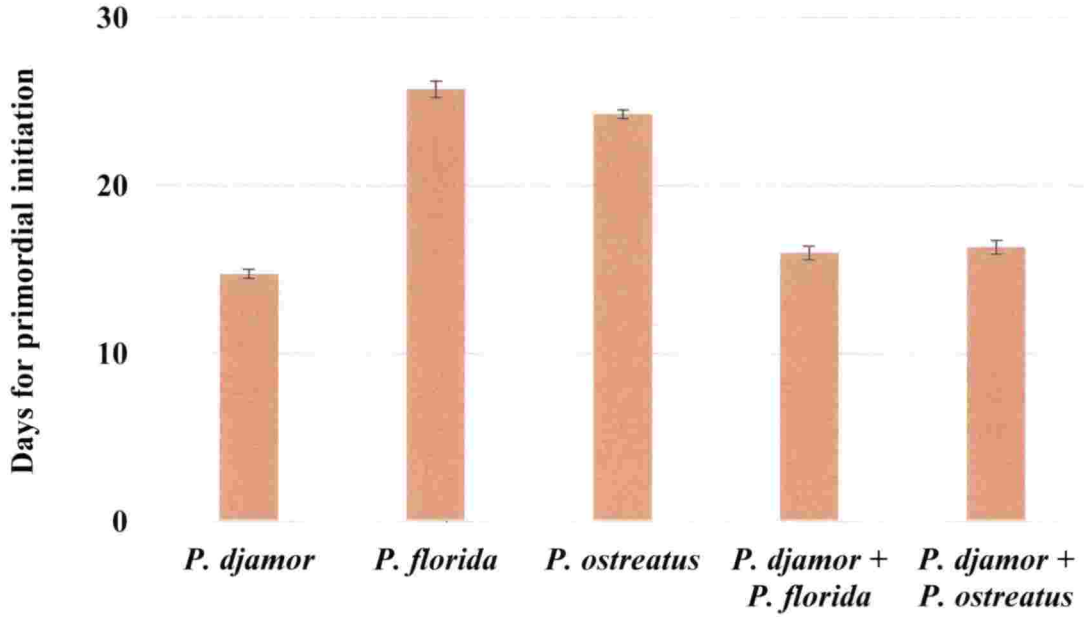


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Figure 4. Comparison of days required for primordial initiation in species of *Pleurotus* and their mixed spawn

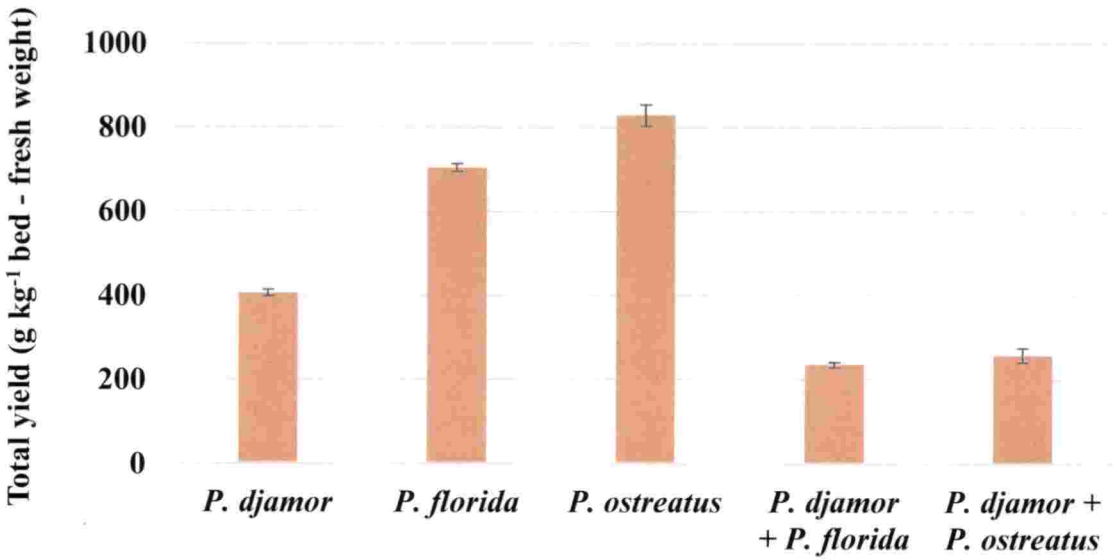

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Figure 5. Yield comparison of species of *Pleurotus* and their mixed spawn

migration between two dikaryotic mycelia. Reduced BE and early decomposition of the mixed spawn beds could be due to inter-strain competition or lethal fusion at the region of mating and difference in the growth stages of the mycelium of the parents (Anitha, 1998).

5.4 STUDIES ON STRAIN IMPROVEMENT BY HYBRIDISATION THROUGH CROSSING OF SINGLE SPORE CULTURE

5.4.1 Preparation of Monosporous Culture

Tissue cultured isolates are needed to screen in large numbers to obtain a desirable culture, and they neither represent the true product of meiosis nor are homokaryotic, thus are unsuitable for breeding material for genetic improvement. Initial selection of strain of interest and subsequent breeding for strain improvement require genetically pure culture and that should represent the result of a true meiotic event. This suggests the isolation of individual basidiospores, which is one among the most important steps to develop homokaryons.

Basidiospores of species of *Pleurotus* were isolated based on serial dilution method demonstrated by Bahukandi and Sharma (2002). Twenty marked germinated spores from each isolates were picked up using a sterile inoculation needle and were allowed to grow in PDPA media. Initial screening mechanism is necessary to restrict number of cultures for field evaluation. Thus single spore cultures were observed for its mycelial appearance and radial growth rate. As observed by Bahukhandi and Sharma (2002), monokaryon had slower growth rate compared to the dikaryon and in some case, the growth was limited to inoculum. Since the performance of the dikaryon depends on the monokaryon, selection of desirable single spore culture is essential. Ten fast growing, dense and cottony single spore cultures were selected from each strain. All the other slow growing and fluffy cultures were screened out (Gharehaghaji *et al.*, 2007; Guadarrama-Mendoza *et al.*, 2014).

Single spore cultures are unable to produce sporocarps and could yield fruiting bodies only after mating with desirable homokaryons. It lacks clamp connection and that was further confirmed by observing it under microscope.

5.4.2 Cross Breeding of Monokaryotic Cultures

The major objective of hybridization was to induce variability in the existing germplasm by combining desirable characteristics from different strains. This could be achieved by pairing monosporic cultures. The single spore cultures of *P. djamor* were tested for compatibility in every possible combination with *P. florida* and *P. ostreatus* separately. Formation of thick strand at the interaction zone of the monokaryotic culture implies the successful mating. Bahukandi and Sharma (2002) inferred that, among the four basidiospores produced by *Pleurotus,* one type can only mate with one of the remaining three spores. Thus the sterile fertile ratio would be 75:25. Mating type in heterokaryotic mushrooms like *Pleurotus* is controlled by two genetic loci named as A and B. The dikaryon was generated only when anastomosis involved haploids heteroallelic at both the loci (Jaswal *et al.,* 2013).

Based on the prominent interaction and formation of thick tuft in the contact zone, ten such compatible mating pairs were selected. Most of the other remaining pairs did not show compatibility. The crossing pair exhibited inhibition, where one mycelium grew faster and surrounded the other. This is due to secretion and diffusion of inhibitory substances (Esser and Blaich, 1994). Other crossing resulted in mutual repulsion due to antagonistic reaction. The incompatibility of monosporous cultures may also be due to the lab conditions which hinder compatible mating (Ikeda *et al.*, 2002) *i.e.,* the culturing environment might not be suitable for the hybridization condition. These lacked clamp connection due to the absence of nuclear migration during mating.

A small portion of mycelium was cut from the junction and observed under microscope for clamp connection, since the formation of heterokaryon was restricted to the contact zone (Gharehaghaji *et al.,* 2007). Thick stranded fast

growing six dikaryon cultures were selected for the study and spawn production. Fully grown spawn was then used for mushroom bed preparation and the performance was recorded. Among the six dikaryons, two cultures of *P. djamor* x *P. florida* and three cultures of *P. djamor* x *P. ostreatus* were found to be the best in yield attributes. Both hybrids produced sporocarps with intermediate characters and increased yield. The selected hybrids were then subjected to stability studies for three generation and the best hybrid from each of the above was selected.

Hybrids along with the parents were compared for their growth performance. Both the hybrids recorded earliness in mother spawn production over both the parents *i.e.,* 8.5 and 8.75 days respectively for the hybrids of *P. djamor* x *P. ostreatus* and *P. djamor* x *P. florida.* Similarly, the number of days needed for complete spawn run, primordial initiation and first harvest were also minimum for these hybrids. The days required for primordial initiation were reduced in hybrid of *P. djamor* x *P. florida* by two and 13 days respectively than the parents; and three and 12 days in hybrid of *P. djamor* x *P. ostreatus*, compared to its respective parents (Figure 6). The faster radial growth rate of mushroom mycelia and better colonisation are the morphological markers of a worthy mushroom strain (Gupta *et al.*, 2011). The faster substrate colonization leading to rapid completion of the crop cycle, will accelerate the time to initiate primordial formation (Yang *et al.*, 2013). The thicker mycelium mat provides better ability to colonize vast agricultural lignocellulosic wastes, as Abdulgani *et al.* (2017) observed in the hybrid of *P. pulmonarius* and *P. citrinopileatus,* which completed its spawn run earlier compared to the two parental strains.

The average sporocarp weight of the hybrids was intermediate to their parents. Total crop period of the two hybrids (*P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus*) was reduced which was comparable with *P. djamor*. The BE of the hybrids *viz., P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus* was improved by 34.38 per cent and 48.48 per cent respectively over the parent, *P. djamor* (Figure 7). Prasad (2008) reported that all the hybrids formed from

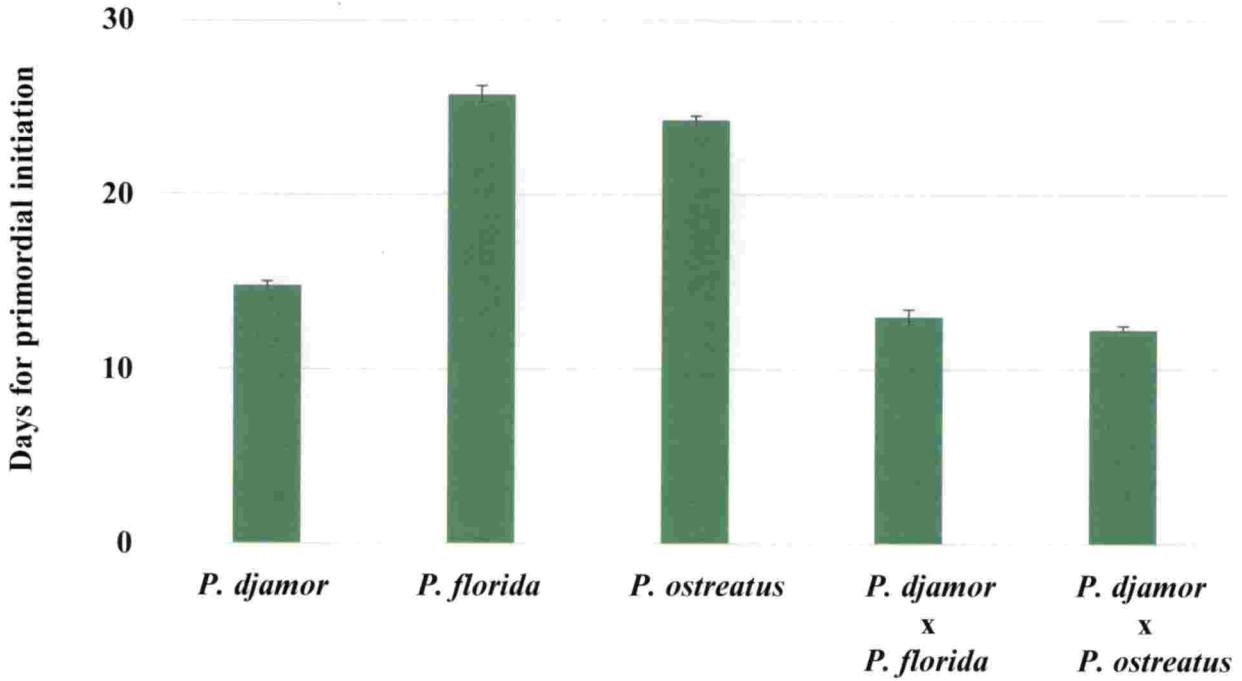

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Figure 6. Comparison of days required for primordial initiation in species of *Pleurotus* and their hybrids

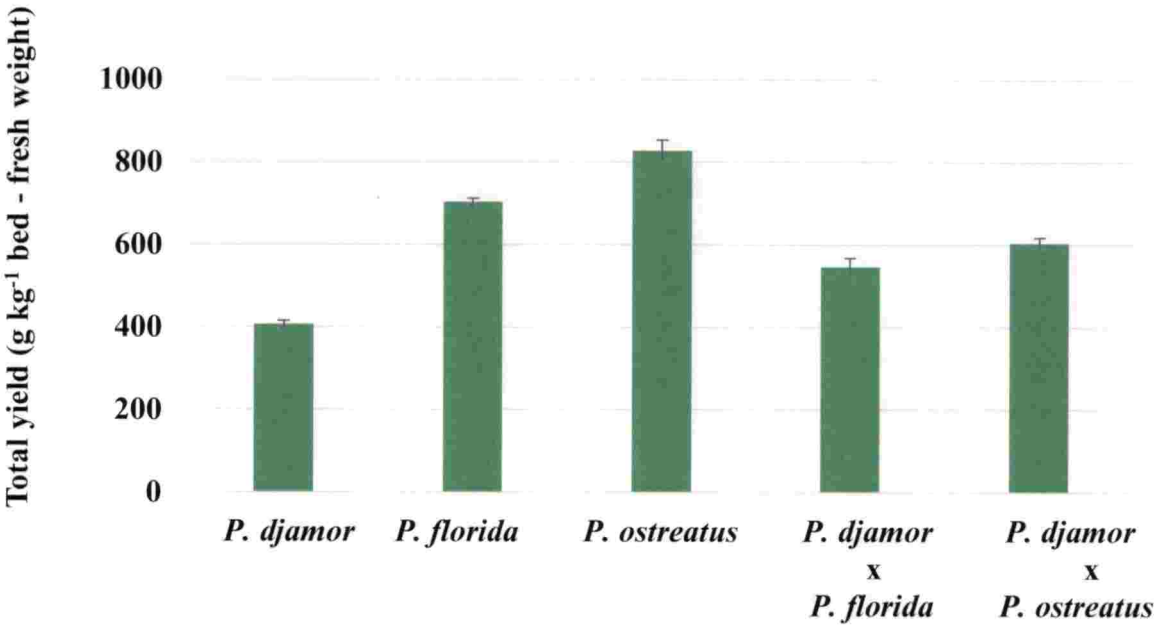

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Figure 7. Yield comparison of species of *Pleurotus* and their hybrids

P. sajor-caju x *P.djamor*, *P. sajor-caju* x *P. florida*, *P. sajor-caju* x *P.flabellatus* and *P. florida* x *H. ulmarius* had significantly superior yield compared to their respective high yielding parents.

The hybrids and their parents were studied for the morphological characteristics and the hybrids produced desirable phenotypic characters. Hybrids produced light pink coloured slightly delicate fruiting bodies which were intermediate to pink coloured leathery *P. djamor* and white coloured delicate *P. florida* and *P. ostreatus*. The pileus dimension of the hybrids was also intermediate to their parents. Similarly, the length of the stipe increased in both hybrids over *P. djamor*. This is in accordance with the study of Bahukhandi and Sharma (2002) who observed the blended characters in hybrids when crossing was done between *P. sajor-caju* and *P. cornucopiae*. The shape and size of the fruiting body was similar to *P. sajor-caju* while the colour was white resembling *P. cornucopiae*. Sawashe and Sawant (2005) observed that the average size of the hybrid fruiting bodies formed by crossing *P. eous* with *P. florida* was intermediate of both the parents, while the stipe length and average weight increased with respect to *P. eous* but on par with *P. florida*. Abdulgani *et al.* (2017) conducted interspecific hybridization between *P. pulmonarius* and *P. citrinopileatus* and the resultant hybrid had superior sporophore features of *P. pulmonarius* like large fleshy pileus with rigid stipe and cluster-type growing pattern of *P. citrinopileatus*.

The selected monokaryons might have desirable gene or genes from their parents. Thus mycelial mating of monokaryons incited the recombinant strain in the expression of phenotypic characters which were not present in the parental dikaryon (Guadarrama-Mendoza *et al.*, 2014). The enhancement in yield and nutrient status may be due to genetic change of hybrids by crossing (Heera, 2006)

Based on the comparative performance of hybrids with their parents, *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus* was found to be promising with respect to increased BE over *P. djamor* with reduced crop period than the

parents. Hybrids were found to be superior to their parents with improved pileus size and slightly delicate texture favouring good transit, packaging and consumer preference.

5.5 STUDIES ON STRAIN IMPROVEMENT BY GAMMA IRRADIATION

Radiation induced mutation is the widely used method to directly improve strains compared to selection and hybridisation which are laborious and time consuming. Gamma radiation is an effective ionizing radiation, which has the ability to penetrate better into the cell walls of mushroom mycelia and target cells (Esser, 1971).

The cultures of the three isolates were exposed to gamma irradiation at 20 and 25 Gy from Radio Tracer Laboratory, Kerala Agricultural University, Thrissur. Mushroom cells are composed of vesicles secreting many types of enzymes to support the growth at tip of mycelia. Hyphal tips have high metabolic rate due to extensive division on those cells to produce nuclei for the newly formed cell compartments (Anitha, 1998). Thus precaution was taken to gamma irradiate on undifferentiated hyphal cells, having high radiosensitivity, to produce maximum effect.

The resultant mutants were subjected to cultivation trials and the screening was done based on the superior quality attributes. The selected mutants were studied for three successive generation to ensure the stability. Comparative performance of the parental isolates along with the selected mutants was recorded (Figure 8, figure 9).

Studies on the effect of gamma irradiation at 20 and 25 Gy on species of *Pleurotus* revealed that *P. djamor* gamma irradiated at 20 Gy yielded maximum with minimum number of days for completing spawn run, pinhead emergence and first harvest. When irradiated at 20 Gy, the BE of irradiated *P. djamor* increased by 9.25 per cent and the days taken for primordial initiation reduced by one day.

Influence of gamma irradiation on *P. florida* was studied and found that *P. florida* gamma irradiated at 25 Gy recorded significantly increased sporocarp size and weight compared to *P. florida* and *P. florida* gamma irradiated at 20 Gy. However, the days needed for pinhead initiation were significantly delayed. Whereas observations on days required for complete spawn run, total crop yield and total crop period of *P. florida* and its mutants did not vary significantly from each other.

P. ostreatus along with their mutants at 20 and 25 Gy were studied for their growth performance. *P. ostreatus* gamma irradiated at 25 Gy exhibited earliness in primordial initiation by three days compared to the parent, *P. ostreatus*. The total crop yield of *P. ostreatus* gamma irradiated at 25 Gy was improved by 12.89 per cent over *P. ostreatus*. Also it produced maximum number of sporocarps within reduced crop period by one week compared to other treatments.

Comparative performance of species of *Pleurotus* with their mutants revealed that *P. ostreatus* gamma irradiated at 25 Gy and *P. djamor* gamma irradiated at 20 Gy were found to be promising. *P. ostreatus* at 25 Gy recorded increased BE of 12.89 per cent within reduced crop period by one week compared to its parent. While *P. djamor* at 20 Gy could enhance earliness in primordial initiation by one day and increased BE of 9.25 per cent over *P. djamor*.

The results are in accordance with Anitha (1998) that gamma radiation in *P. florida* culture at 2 and 2.5 kr level resulted in higher yield than all other levels of irradiation ranging from 0.25 – 2.5 kr. Grodzinskaya and Mikheev (2001) observed 3-5 days acceleration of primordial formation in case of minimum (25 Gy) and maximum (200 Gy) dose of irradiation in *P. ostreatus*. Beejan and Nowbuth (2009) claimed that gamma irradiation did not result in any change in colour of pileus. Rashid *et al.* (2016) found that irradiation can induce more fruiting bodies with no significant difference on size of fruiting bodies. Similar observation was recorded in *P. djamor* irradiated at 20 Gy, but contradictory result on fruiting bodies produced in *P. ostreatus* irradiated at 25 Gy.

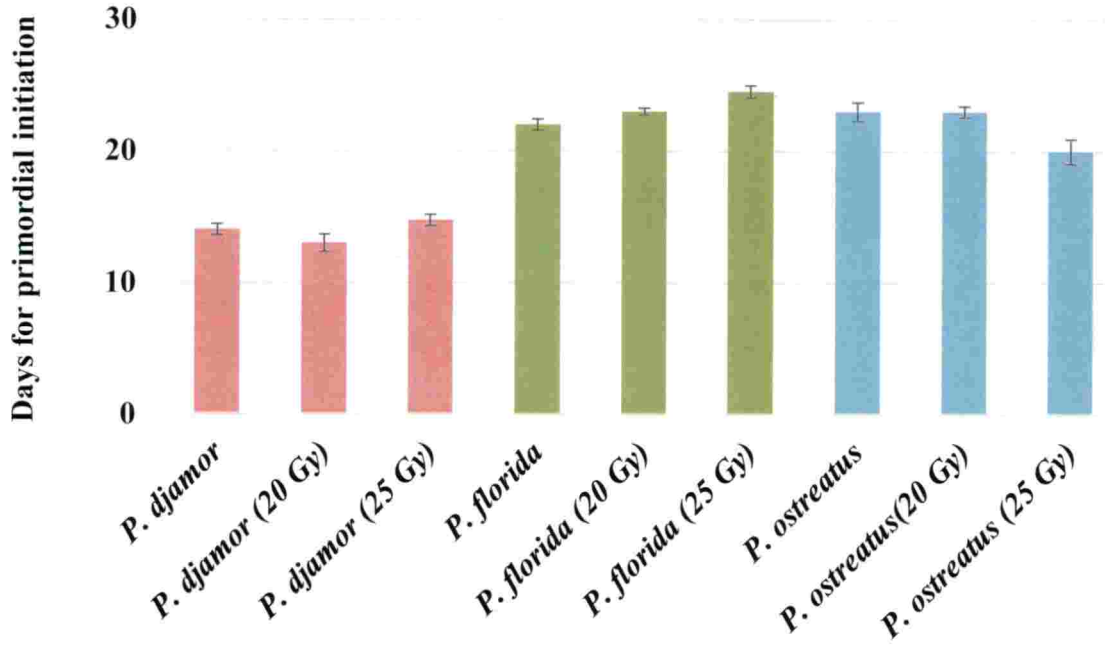

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Figure 8. Comparison of days required for primordial initiation in species of *Pleurotus* and their gamma irradiated (20 and 25 Gy) mutants

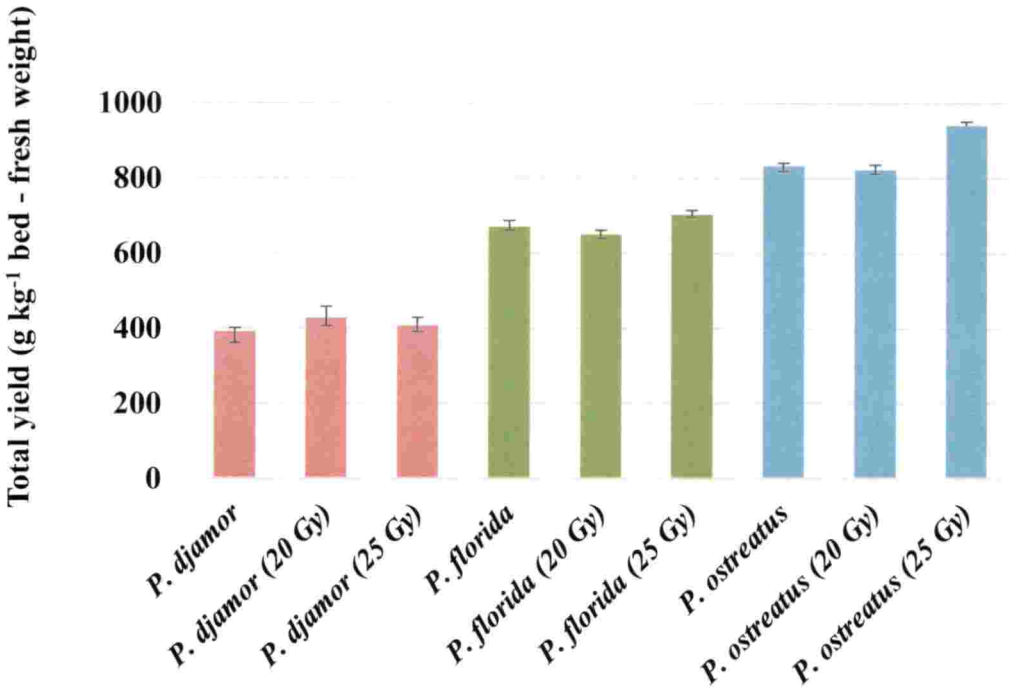

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Figure 9. Yield comparison of species of *Pleurotus* and their gamma irradiated (20 and 25 Gy) mutants

The difference in growth attributes of mutants may be due to the genetic changes caused by gamma radiation. Gamma rays may trigger the expression of recessive genes and produce new genetic variations (Yoon *et al.,* 1990). It can also produce a range of effects on DNA repair mechanism, both through free radical effects and direct action, which include breaks in one or both strands, deletions, duplications, damage or loss of bases and the cross linking of DNA (Kaur *et al.,* 2011).

The variation in impact of gamma irradiation at 20 and 25 Gy on isolates of *Pleurotus* spp. may be due to the difference in sensitivity and subsequent metabolism towards gamma irradiation for each species.

The efficiency of mushroom species in producing fruiting bodies depends on their ability to degrade agricultural substrates through secretion of a variety of hydrolyzing and oxidizing enzymes (Zhu *et al.,* 2013). The enzymes facilitate in utilizing insoluble lignocellulosic substrates and all kinds of carbon sources in the substrate used, resulting in the production of fruiting bodies (Henry and Rajakumar, 2013). The increased rate of mycelial ramification and improved BE may be due to enhanced cellulolytic activity developed through radiation mutagenesis. Lee *et al.* (2000) reported that cellulolytic activity of *P. ostreatus* increased by ten times higher than the control when the mycelia was irradiated at 1-2kGy. Similarly, a potent cellulase mutant of *P. florida* induced by gamma radiation at a dose of 0.51 kGy exhibited 17.24 per cent more cellulolytic activity than the wild type (Prabhu and Young-Keun, 2016)

5.6 COMPARISON OF THE BEST CULTURES

5.6.1 Yield

The best improved cultures from the above mentioned experiments along with their native isolates were used for their comparative performance. The selected cultures were hybrids of *P. djamor* x *P. florida*, *P. djamor* x *P. ostreatus*, *P. djamor* gamma irradiated at 20 Gy, *P. ostreatus* gamma irradiated at 25 Gy. The BE of all the treatments were compared and found that *P. ostreatus*

gamma irradiated at 25 Gy recorded highest BE with 12.3 per cent over *P. ostreatus*. Similar results were also reported by Djajanegara and Harsoyo, (2008) that significantly higher productivity in mutant strain of oyster mushroom irradiated at 0.75 kGy compared to control due to mutation of genes within cell or change in repair mechanism of DNA. The hybrids were found to be superior to their parents with reference to increased BE with reduced crop period. Increased BE over *P. djamor* was recorded for both the hybrids. Among hybrids *P. djamor* x *P. ostreatus* had higher yield potential than *P. djamor* x *P. florida*. The hybrids, *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus* had reduced crop period compared to *P. florida* and *P. ostreatus* respectively. The phenotypic characters may be changed due to the mycelial mating of monokaryons in the recombinant strain, resulting in genetic change of hybrids (Guadarrama-Mendoza *et al.*, 2014).

From this study, *P. djamor* x *P. ostreatus* and *P. ostreatus* gamma irradiated at 25 Gy ensure an excellent BE with reduced crop period, thus they could make wide acceptance among mushroom growers as these criteria promise cost-effectiveness and higher income.

5.6.2 Proximate Constituents in Species of *Pleurotus*

The nutrient analysis of three *Pleurotus* spp. along with *P. djamor* x *P. florida*, *P. djamor* x *P. ostreatus* and *P. ostreatus* gamma irradiated at 25 Gy was carried out (Figure 10). Moisture content, protein, carbohydrate and crude fibre were analysed. Apart from BE, texture and flavour, mushrooms are also appreciated for their nutritive characteristics (Manzi *et al.,* 2001).

5.6.2.1 Estimation of Moisture Content

Fresh mushrooms generally have high moisture content (Adebayo *et al.,* 2011). Moisture content in mushroom is highly influenced by stage of harvest, strain, growing environment and post harvest environment (Ahmed *et al.,* 2013).

Highest moisture content was recorded for *P. florida* (92.16 %) (Ahmed *et al.*, 2009; Maftoun *et al.*, 2015) and least moisture content for *P. djamor* (85.97 %). The moisture content of the hybrids, *P. djamor* x *P. florida*

(87.81 %) and *P. djamor* x *P. ostreatus* (88.31 %) was intermediate to the parents. Selvakumar *et al.* (2015) reported moisture content of *P. ostreatus* var. *florida* as 83.18 per cent and *P. djamor* var. *roseus* as 79.52 per cent.

5.6.2.2 Estimation of Carbohydrate

Carbohydrate content represents the major portion of the dried mushroom sample. *P. ostreatus* gamma irradiated at 25 Gy (56.33 %) recorded highest carbohydrate content followed by *P. ostreatus* (55.77 %). Least content was reported in *P. djamor* (45.79 %). Hybrids of *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus* had carbohydrate content superior to *P. djamor*. *P. florida* had 52.42 per cent carbohydrate content. The carbohybrate content of *P. florida* reported by other workers, Ahmed *et al.* (2009) and Maftoun *et al.* (2015) were similar with the result recorded. Ashraf *et al.* (2013) recorded 37.69 per cent carbohydrate content in *P. djamor*.

5.6.2.3 Estimation of Protein

The protein content of the hybrids, *P. djamor* x *P. florida* (26.54 %) and *P. djamor* x *P. ostreatus* (25.14 %) were significantly superior to both their parents. Whereas *P. ostreatus* gamma irradiated at 25 Gy (21.47 %) did not vary with the parent (22.31 %) in protein content. Roy *et al.* (2000) inferred that gamma induced mutants of *Pleurotus* spp. resulted in reduced spawn run time and stimulated yield without any effect on protein and carbohydrate content. The results are in agreement with Selvakumar *et al.* (2015) who reported that maximum protein content was found for the hybrid of *P. ostreatus* var. *florida* and *P. djamor* var. *roseus*, compared to the parents.

5.6.2.4 Estimation of Crude Fibre

The dietary fibre content was found in high levels in *P. djamor* x *P. florida* (14.07 %), followed by *P. djamor* x *P. ostreatus* (13.35 %). The least content was recorded in *P. florida* (8.38 %). Maftoun *et al.* (2015) reported that the fibre content of *P. florida* (11.5 %) was less compared to *P. djamor* (17.2 %).

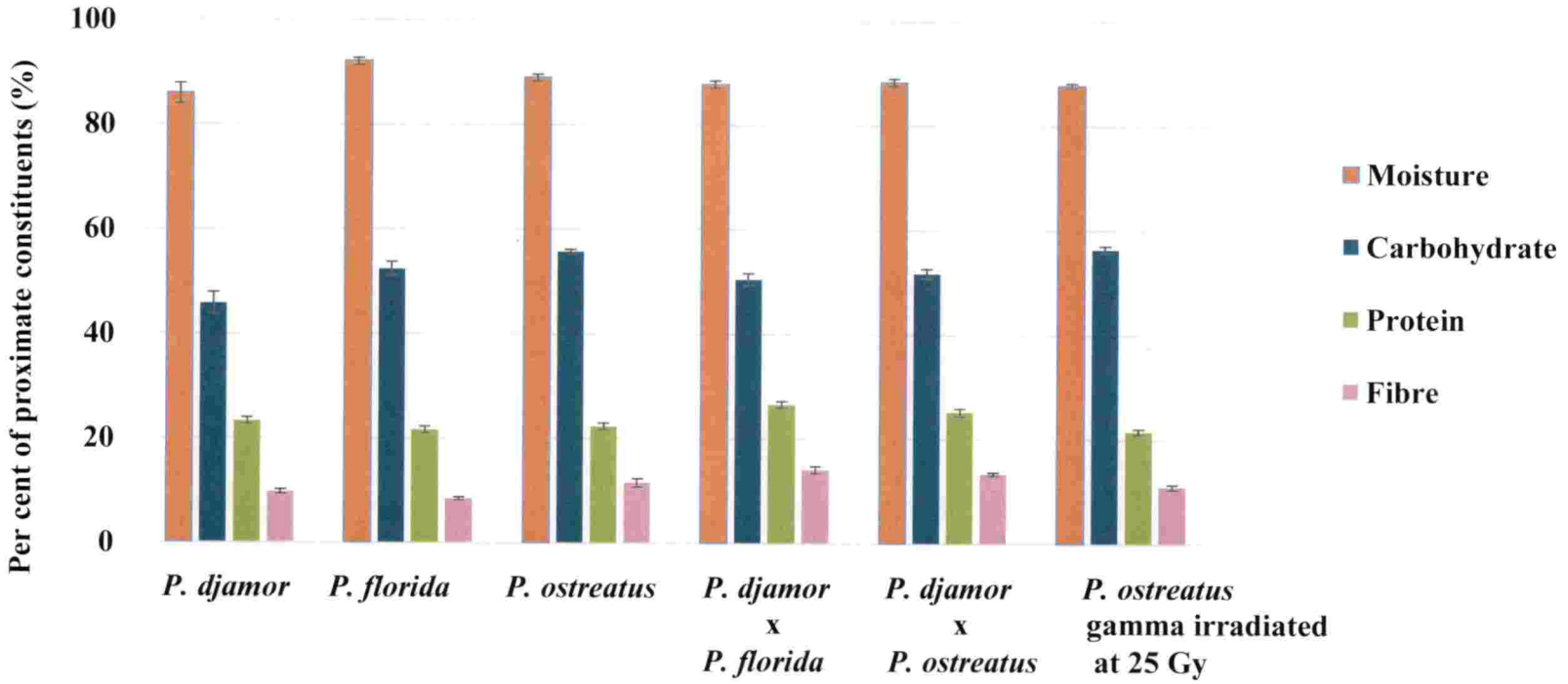

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Figure 10. Comparison of moisture (fresh weight), carbohydrate (dry weight), protein (dry weight) and fibre content (dry weight) in species of *Pleurotus* and their improved strains developed through hybridisation and gamma irradiation followed by three generation of selection

Selvakumar *et al.* (2015) obtained intermediate fibre content for the hybrid between *P. ostreatus* var. *florida* and *P. djamor* var. *roseus*.

Higher per cent of protein and crude fibre content on dry weight basis for hybrids may be due to the respective genes interaction and the effect can be considered as heterobeltosis.

5.7 PEST AND DISEASE INCIDENCE

The major pests observed during mushroom cultivation were springtail, phorid flies and staphilinid beetle. Most predominant pest noted among the trials was springtails (*Seira* sp.). Along with pests, the competitor weeds observed on mushroom beds also caused significant yield reduction. These were *Coprinus* sp., *Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp., and *Chaetomium* sp. Among the contaminants *Trichoderma* spp. caused the significant loss. In confirmation with the above observations, Kumar and Sarathi (2017) and Jose (2018) also observed the same pests and competitor weeds on mushroom beds of *Pleurotus*.

5.8 RAPD ANALYSIS

RAPD marker is preferred over other molecular markers for the genetic diversity analysis as it is simple, easy to use and does not require any prior knowledge of the DNA sequence (Dwivedi *et al.*, 2018).

5.8.1 DNA Isolation

Genomic diversity of parents and improved strains of *Pleurotus* spp. was investigated by RAPD analysis. DNA of the parents *P. djamor*, *P. florida*, *P. ostreatus* and their improved strains *P. djamor* x *P. florida*, *P. djamor* x *P. ostreatus*, *P. djamor* gamma irradiated at 20 Gy, *P. florida* and *P. ostreatus* gamma irradiated at 25 Gy were isolated using DNeasy plant mini kit (Qiagens).

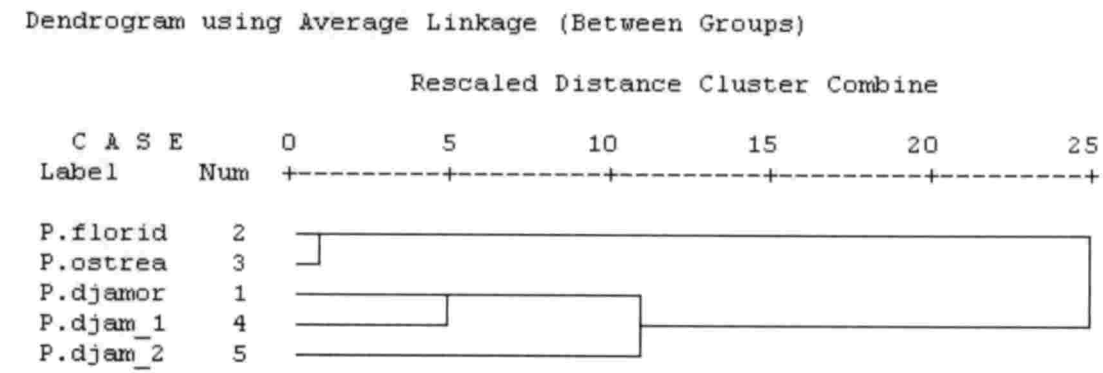
5.8.2 PCR Amplification

The isolated DNA of parents and their hybrids were amplified using RAPD primer OPT 5 (GGGTTTGGCA), based on the earlier reports of Agarwal *et al.* (2013).

Based on the presence and absence of amplicons, number of monomorphic and polymorphic bands were recorded. Monomorphic bands are those which are present in both parents and their hybrids; polymorphic bands are present in one or more, but not in all individuals. The polymorphisms observed between the parents are used as markers for hybrid identification (Ali *et al.,* 2008).

The primer OPT 5 amplified a total of 23 scorable bands and the size of the amplified products varied from 200 bp to 1000 bp. Out of total amplicons produced, three amplicons were monomorphic for the parent, *P. florida* and *P. djamor* x *P. florida* while four amplicons were monomorphic for the parent, *P. djamor* and *P. djamor* x *P. florida*. The bands common in hybrids and either of the parent are especially important marker to identify the true hybrid (Akhare *et al.*, 2008). All the other bands were polymorphic. Similarly, four and two amplicons were monomorphic for *P. djamor* and *P. ostreatus* respectively with *P. djamor* x *P. ostreatus*. Almost all RAPD bands from both parents were found in RAPD profiles of the hybrids. This indicated the high penetrance and dominant nature of RAPD markers.

A typical RAPD reaction produces multiple amplification products and each band was assumed to represent a unique genetic locus (Mishra *et al.,* 2012). Statistical analysis was done for the bivariate data obtained with the primers to generate Jaccard's similarity coefficient. The statistical analysis of the RAPD data revealed the genetic relatedness and the distance between the parents and hybrids. UPGMA based dendrogram exhibited two major groups in which hybrids formed a single cluster with *P. djamor* (Figure 11). Among the hybrids, *P. djamor* x *P. florida* exhibited more similarity to *P. djamor*. Abdulgani *et al.* (2017) carried out hybridisation between *P. pulmonarius* and *P. citrinopileatus*. Further analysis



Abbreviated Name	Extended Name
P.djam_1	P.djamorXP.florida
P.djam_2	P.djamorXP.ostreatus
P.florid	P.florida
P.ostrea	P.ostreatus

The scale is based on Jaccard's co-efficient of similarity

Figure 11. Dendrogram of species of *Pleurotus* and their hybrids constructed by UPGMA of binary matrix obtained from RAPD

via RAPD confirmed that the five hybrids developed exhibited high genetic homology with *P. pulmonarius*.

The isolated DNA of parents and their mutants were amplified using OPS 5 (TTTGGGGCCT), based on the earlier reports of Agarwal *et al.* (2013). The RAPD pattern of OPS 5 generated 41 amplicons with the molecular weight ranged from 400 to 1500 bp. Two amplicons present in mutants of *P. florida* gamma irradiated at 25 Gy and *P. ostreatus* gamma irradiated at 25 Gy were absent in their respective parents. While one polymorphic amplicon present in *P. djamor* was absent in its mutant at 20 Gy. The genetic similarity of the mutants *viz.*, *P. djamor* at 20 Gy, *P. florida* at 25 Gy and *P. ostreatus* at 25 Gy decreased to 83.3, 75 and 78 per cent compared to their respective parents. The RAPD patterns of the control and five strains (PO-5, PO-6, PO14, PO-15 and PO-16) of *P. ostreatus* gamma irradiated at 1 and 2 kGy were analysed by Lee *et al.* (2000). The genetic similarities of the three strains PO-5, PO-6 and PO-14 decreased to 93, 91 and 73 per cent of the control, respectively. The genetic similarities of the two strains PO-15 and PO-16 from 1 kGy re-irradiation group of PO-14, decreased to 91 and 82 per cent of PO-14 strain and to 77 and 64 per cent of the control, respectively.

Mutation could induce changes in genome sequence which effect primer annealing sites and this resulted in polymorphism in RAPD profile. The polymorphism produced by RAPD primers may be due to the base substitution, insertion and deletion of the genetic material (Chopra, 2005; Jusuf, 2010). In this way the RAPD analysis can provide a simple and reliable method for measuring genomic variation.

The disappearance of normal bands in mutant may be due to DNA damage, DNA protein cross links, point mutation or complex chromosomal rearrangement induced by gamma irradiation. Appearance of new PCR products detected in RAPD analysis revealed a change in some oligonucleotide priming sites due to mutations (Dhakshanamoorthy *et al.*, 2011)

The primers tested produced clear and reproducible amplicons. This is in accordance with Liu (1999) who inferred that RAPD markers were highly reproducible in size range from 200 to 1500 base pairs. Thus RAPD analysis is a sensitive and powerful tool for genetic variation assessment at DNA level among *Pleurotus* spp. (Yadav *et al.*, 2017).

Summary

6. SUMMARY

The present research project was aimed to develop improved strain of *Pleurotus* sp. from native isolates *viz.*, isolate 1 (*P. djamor),* isolate 2 (*P. florida*) and isolate 3 (isolate with 80 per cent similarity to *P. florida*). Isolate 3 was later identified as *P. ostreatus* through ITS sequencing. The cultures of isolates were obtained from mushroom unit of Instructional Farm, College of Agriculture, Vellayani. The cultures were made to spawn and cultivated on paddy straw substrate.

P. florida and *P. ostreatus* produced comparatively larger sporocarps with long stipe and higher carp weight than *P. djamor*. *P. djamor*, *P. florida* and *P. ostreatus* produced pink, white and greyish white colour fruiting bodies respectively. *P. djamor* and *P. florida* attained harvesting maturity in three days from pinhead emergence, whereas *P. ostreatus* required four days. Spore prints of *P. florida* and *P. ostreatus* were white in colour, while spore print colour of *P. djamor* was light pink to creamish white. The mycelia of *Pleurotus* spp. were septate and with clamp connections. The basidiospores of *Pleurotus* spp. were cylindrical and produced on tetra-sterigmatic clavate basidia present on the hymenium of the sporocarps. Subventricose to clavate shaped cheilocystidia were observed in all the *Pleurotus* spp. studied.

Pest and disease free and partially matured healthy sporocarps of the three *Pleurotus* spp. were tissue cultured in PDPA medium. The culture was further purified by hyphal tip method and morphological characters of the cultures were studied. *P. djamor* produced cream coloured thick cottony mycelium with concentric pattern. White fluffy mycelial growth with even margin was observed in *P. florida* whereas thick stranded greyish white mycelium with radiating margin was observed in *P. ostreatus*. *P. florida* recorded faster mycelial growth and completed the growth in 9 cm (diameter) petri plate in nine days and it was followed by *P. ostreatus* (10 days) and *P. djamor* (12 days).

Strain improvement programme was conducted by spawn mixing, hybridisation through crossing of single spore cultures and gamma irradiation. Spawn of two different species *viz.*, *P. djamor* + *P. florida* and *P. djamor* + *P. ostreatus*, were mixed and cultivation trials were conducted. For the given *Pleurotus* spp., hybridization or crossing of isolates did not take place by spawn mixing, thus did not have any significance in strain improvement. The beds of mixed spawn produced sporocarps of both parents separately. BE of *P. djamor* + *P. florida* was reduced by 41.95 per cent and 66.49 per cent respectively, compared to their parents. Similarly, BE of *P. djamor* + *P. ostreatus* was low compared to their parents by 36.41 per cent and 68.81 per cent respectively.

Hybridization was carried out between the compatible single spore cultures of *P. djamor* with *P. florida* and *P. ostreatus* independently by dual culture method. The hybrids were confirmed by the presence of clamp connection in mycelia from the interaction zone. Both the hybrids, *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus* completed mycelial run in spawn in minimum number of days *viz.,* 8.75 and 8.50 respectively. This was followed by *P. djamor*, *P. florida* and *P. ostreatus* which completed the mycelial run in spawn by 15.75, 20.75 and 20.50 days respectively. The BE of the hybrids *viz., P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus*, was improved by 34.38 per cent and 48.48 per cent respectively over the parent, *P. djamor*. The days needed for primordial initiation were significantly reduced in the hybrids of *P. djamor* x *P. florida* by two and 13 days than the parents, *P. djamor* and *P. florida*. Whereas the days were reduced by three and 12 days in hybrids of *P. djamor* x *P. ostreatus*, compared to its respective parents. Blended characters of both parents were observed in the hybrids. Hybrids produced light pink coloured slightly delicate fruiting bodies, which was intermediate to pink coloured leathery *P. djamor* and white coloured delicate *P. florida* and *P. ostreatus*. Slightly delicate texture of the hybrids favours good transit, packaging and consumer preference. The pileus dimension of the hybrids was also intermediate to their parents. Sporocarps with wavy margin and stout stipe over *P. djamor* were produced by the hybrids of *P. djamor* x *P. florida*. Whereas, sporocarps with slight depression towards the base and

increased stipe length over *P. djamor* were produced by the hybrids of *P. djamor* x *P. ostreatus*.

The cultures of the three isolates were gamma irradiated at 20 and 25 Gy doses in Radio Tracer Laboratory, Kerala Agricultural University, Thrissur. Days needed for pinhead emergence significantly reduced when *P. djamor* was gamma irradiated at 20 Gy (13 days) compared to *P. djamor* (14 days) and *P. djamor* gamma irradiated at 25 Gy (14.60 days). Total yield of *P. djamor* gamma irradiated at 20 Gy improved by 9.25 per cent over *P. djamor*.

P. florida gamma irradiated at 25 Gy recorded significantly increased sporocarp size and weight compared to *P. florida* and *P. florida* gamma irradiated at 20 Gy. However, the days needed for pinhead initiation was significantly delayed (24.40 days) in *P. florida* gamma irradiated at 25 Gy compared to *P. florida* (22 days).

P. ostreatus and its gamma irradiated mutants at 20 and 25 Gy were studied. Earliness in primordial initiation by three days compared to the parental *P. ostreatus* was observed in *P. ostreatus* gamma irradiated at 25 Gy. The total crop yield of *P. ostreatus* gamma irradiated at 25 Gy was improved by 12.89 per cent over *P. ostreatus*. Compared to *P. ostreatus*, the total crop period reduced by one week when it was gamma irradiated at 25 Gy. The mutant of *P. ostreatus* at 25 Gy recorded maximum number of sporocarps (124) compared to *P. ostreatus* gamma irradiated at 20 Gy (103.75) and *P. ostreatus* (101.75).

The best improved cultures from the above mentioned experiments along with their native isolates were cultivated on paddy straw substrate and the performance was compared. The selected cultures were hybrids of *P. djamor* x *P. florida*, *P. djamor* x *P. ostreatus*, *P. djamor* gamma irradiated at 20 Gy and *P. ostreatus* gamma irradiated at 25 Gy. *P. ostreatus* gamma irradiated at 25 Gy recorded highest BE with 12.30 per cent increase over *P. ostreatus*. The hybrids were superior to their parents with reference to increased BE with reduced crop period. Both the hybrids recorded increased BE over *P. djamor*. Among hybrids,

P. djamor x *P. ostreatus* had higher yield potential than *P. djamor* x *P. florida*. Crop period was reduced in both hybrids of *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus* compared to their parents.

The nutrient analysis of three *Pleurotus* spp. along with *P. djamor* x *P. florida*, *P. djamor* x *P. ostreatus* and *P. ostreatus* gamma irradiated at 25 Gy was carried out. The moisture content of the hybrids of *P. djamor* x *P. florida* (87.81%) and *P. djamor* x *P. ostreatus* (88.31%) was intermediate to their parents. *P. ostreatus* gamma irradiated at 25 Gy (56.33%) recorded highest carbohydrate content followed by *P. ostreatus* (55.77 %). Improved carbohydrate content over *P. djamor* was recorded in hybrids of *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus*. The protein content of the hybrids of *P. djamor* x *P. florida* (26.54 %) and *P. djamor* x *P. ostreatus* (25.14 %) were superior to both their parents. The hybrids, *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus*, also recorded maximum fibre content *viz.,* 14.07 per cent and 13.35 per cent respectively.

The major pests observed during mushroom cultivation were springtail, phorid flies and staphilinid beetle. Most predominant pest documented was springtails (*Seira* sp.). Along with pests, the competitor fungi observed in mushroom beds viz. *Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp., *Coprinus* sp., and *Chaetomium* sp. also resulted in significant yield reduction.

Molecular characterisation of the improved strains, *viz., P. djamor* x *P. florida*, *P. djamor* x *P. ostreatus*, *P. djamor* gamma irradiated at 20 Gy, *P. florida* and *P. ostreatus* gamma irradiated at 25 Gy was done using RAPD. Based on the presence or absence of amplicons, the bands were scored. The bivariate data obtained was analysed using SPSS software to generate Jaccard's similarity coefficient. The pair wise coefficient values of the hybrids along with parents, ranged from 0.143 to 0.750. The hybrid, *P. djamor* x *P. florida* had 66.70 per cent and 50 per cent similarity to *P. djamor* and *P. florida* respectively. While *P. djamor* x *P. ostreatus* had 57.1 per cent similarity to *P. djamor* and 28.6 per cent similarity to *P. ostreatus*. The matrix was subjected to unweighed pair group

method analysis to develop dendrogram. The hybrids formed a single cluster with *P. djamor*. The distance matrix computed for the better mutant, *P. ostreatus* gamma irradiated at 25 Gy indicated 22 per cent polymorphism with the parent *P. ostreatus*.

The present study demonstrated the exploitability of two promising strains viz. *P. djamor* x *P. ostreatus* and *P. ostreatus* gamma irradiated at 25 Gy, for further field level, multiseasonal and multilocation studies. The hybrid of *P. djamor* and *P. ostreatus* could be preferred for the production of sporocarps with favourably blended characters and increased BE with reduced crop period compared to both the parents. *P. ostreatus* gamma irradiated at 25 Gy, which recorded increased BE with reduced crop period by one week compared to the parent, may be subjected to further studies. Prior to recommending for large scale cultivation, both the improved strains need to be subjected to multilocation and multiseason trials.

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Appendices

APPENDIX I

Data sheet

Sl. No.: Date of collection :

Collected by : Locality :

(Village/ Taluk/ Dist)

Final identification :

(Confirmed by .....)

Taxonomy ;

Order :

Family ;

GENERAL

Common name : Local name:

Soil type : Vegetation :

Substrate :

Season : Temp : R. H : Rainfall :

Any other information on climate :

Others :

Habit : Terrestrial / Lignicolous/ Epixylose / Coprophilous / Humicolous

Habitat ; Solitary / Scattered / Caesipitose / Gregarious

PILEUS

When young : Conical / spherical / Campanulate / convex

Shape :

At maturity : Infundibuliform / Umbonate / Broadly umbonate /
Campanulate/ Umbilicate / Aplanate / Conical/

Petaloid/ Flabelliform/ Mucronate / Depressed / Dimidate / Resupinate

When young :

Size :

At maturity :

Colour : .....

Texture : Soft/ Brittle/ Fleshy/ Coriaceous/ Hyphanous/ Fragile/ Cartilaginous/ Membraneous

Surface : Smooth/ Scaly/ Rugose/ Rugulose/ Viscid/ Striate/ Dry/ Squamulose/ Velutinous/ Pubescent/ Strigose/ Sulcate/ Tomentose/ Aveolate/ Farinose/ Floccose/ Punctate/ Rivose/ Rivulose

Margin : Serrate/ Serrulate/ smooth/ Undulate/ Reflexed/ Involute/ Fimbriate/ Incised/ Lobed/ Revolute

Context colour:

Before cutting : .....

After cutting : .....

Colour changes with :-

Melzer's reagent : Amyloid/ Psuedoamyloid/ Inamyloid

Green Vitrial : .....

Phenol : .....

Sulphovanilin : .....

GILLS

Arrangement : Remote/ Free/ Decurrent/ Adanate/ Adnexed/ Sinuate

Shape : Rounded anteriorly or posteriorly/ Lanceolate/ Ventricose/ Reticulate

Texture : Soft/ Brittle/ Ceraceous/ Waxy/ Thick/ Papery/ Opaque

Margin : Smooth/ Wavy/ Serrate/ Fimbriate/ Dentate

Size ; Number /cm

Gill trama : Regular/ Irregular/ Bilateral/ Inverse

Cystidia Size

1. Pilocystidia
2. Pleurocystidia
3. Cheilocystidia
4. Caulocystidia

Shape :

a. Ventricose	b. Clavate
c. Filiform	d. Napiform
e. Lageniform	f. Rostrate
g. Encrusted	h. Ramified
i. Lanceolate	j. Lanceolate
k. Granulate	l. Pointed
m. Beaked	n. Capitate
o. Lacyliform	p. Cylindrical

VEIL

Type : Present / Absent Universal / Partial

Colour :

Texture : Membraneous / Fleshy / Smooth/ Coriaceous

Position :

ANNULUS

Size : .....

Texture : Fleshy / Papery/ Coriaceous/ Thin

Colour : .....

Attachment : Superior broad/Medial pendulous/ Inferior/ Narrow/ Fragments/ Appendiculate/ Fibrillose/ Movable

STIPE

Present (Stipitate) / Absent (Sessile)

Length

Size :

Shape : Clavate/ Obclavate/ Cylindrical/ Solid/ Hollow/ Slender/ Short

Attachment to pileus : Lateral/ Eccentric/Central/ Resupinate/ Glabrous/ Scaly/ Pubescent/ velutinous/ Squamose/ Tomentose

Colour

Before cutting : .....

After cutting : .....

Reaction with Melzer's reagent : Amyloid/ Psuedoamyloid/ Inamyloid

Basal part : Globular/ Annular stipes/ Fusoid/ Bulbous/ Sheathing bulbous/ marginately depressed bulb/Psuedorhizoid/Rhizines/Rhizemorphoid

VOLVA

Present/ Absent Persistent/ Evanescent

Shape : Free/ Lobsed/ Irregular/ Cup like

Colour :

Texture : Soft/ Fleshy/ Tough/ Papery

Odour

Before cutting : .....

After cutting : .....

Taste : Acrid/ Mealy/ Acidulous/ Blunt

SPORE PRINT

Colour : .....

Other details : .....

BASIDIA

Size : .....

Shape : .....

Sterigmata : .....

SPORES

Colour :

Reaction with Melzer's reagent : Amyloid/ Psuedoamyloid/ Inamyloid

Shape : Ovate/ Elliptical/ Globose/ subglobose/ Apiculate/ Cylindrical/ Fusiform/ Angular/ Echinulate/ Verrucose/ Reticulate/ Tuberculate/ Ovoid/ Abtusely fusiform/ Allantoid/ Guttulate/ Pip shaped/ Piriform/ Pedicilate/ Muriform/ Filiform

Other characters of spores : .....

ANY OTHER DETAILS

APPENDIX-1I

Composition of stain used

1. Lactophenol Cotton blue

Phenol crystals	-	20.0 g
Cotton blue	-	0.05 g
Lactic acid	-	20.0 mL
Glycerol	-	20.0 mL
Distilled water	-	20.0 mL

2. Congo Red

Congo Red	-	10.0 g
Distilled water	-	1000 mL

APPENDIX III

Composition of media

1. Potato Dextrose Peptone Agar

Potatoes (Sliced)	-	200.00 g
Agar-agar	-	20.00 g
Dextrose	-	20.00 g
Peptone	-	10 g
Distilled H_20	-	1000 ml

APPENDIX IV

Weather data at AAS, Vellayani, Kerala Agricultural University

Season	Outdoor				Indoor	
	Temp. (°C)		RH (%)	Total rainfall (mm)	Temp. (°C) (9 am)	RH (%)
	Max.	Min.				
November 2018	31.57	23.98	92.08	162.2	25.48	87
December 2018	32	23.48	93.08	51.8	24.32	89
March 2019	34.6	24.8	85.26	0.0	27.26	78

APPENDIX V

Fasta sequence of isolate 3 (*P. ostreatus*)

Forward sequence :

GGAGTTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTTC
AACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTTTCAAGTCGT
CAGACTTGGTTGGCT

Reverse sequence :

ATTTGAGGTCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTG
GACTCTATTCATGCGTGCTATTGATGAGTGATAATTATCACATCATGCG
CAGAGGCAATGAGAAGTCCTGCTAATGCATTTAAGAGGAGCCGACCT
GTCAAGGCCAGCAGCCCCCAACAATCCAAACATCACAATTGGAAAAA
CCCCAGTGAGT

Abstract

DEVELOPMENT OF IMPROVED STRAIN IN OYSTER MUSHROOM (*Pleurotus* spp.)

by

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ABSTRACT

The present study entitled "Development of improved strain in Oyster mushroom (*Pleurotus* spp.)" was carried out in College of Agriculture, Vellayani during 2017-2019 with the objective to develop improved strain of *Pleurotus* sp. from native isolates.

Three native isolates used for the study *viz.,* isolate 1 (*Pleurotus djamor*), isolate 2 (*Pleurotus florida*) and isolate 3 (a native isolate with 80 per cent similarity to *P. florida*), were obtained from Mushroom Unit of Instructional Farm, College of Agriculture, Vellayani. Species level confirmation of isolate 3 was done at molecular level ITS sequencing and was confirmed as *P. ostreatus*.

The morphologic studies of the native isolates revealed that *P. florida* and *P. ostreatus* produced comparatively larger sporocarps with long stipe and higher carp weight than *P. djamor*. *P. djamor*, *P. florida* and *P. ostreatus* produced fruiting bodies with pink, white and greyish white colour respectively. The microscopic characteristics of the isolates were studied. The mycelia were septate and with clamp connections. The basidiospores were cylindrical and produced on tetra-sterigmatic clavate basidia present on the hymenium of the sporocarps.

Strain improvement programme was conducted by spawn mixing, hybridisation through crossing of single spore cultures and gamma irradiation. The experiment was laid out in completely randomised design with required number of replications. Spawn of two different species *viz.*, *P. djamor* + *P. florida* and *P. djamor* + *P. ostreatus*, were mixed and cultivation trials were conducted. For the given *Pleurotus* spp., hybridization or crossing of isolates did not take place by spawn mixing, thus did not have any significance in strain improvement. The beds of mixed spawn produced sporocarps of both parents. In addition, the biological efficiency (BE) of *P. djamor* + *P. florida* was reduced by 41.95 and 66.49 per cent respectively, compared to their parents. Similarly, BE

of *P. djamor* + *P. ostreatus* was lower compared to their parents *viz.*, *P. djamor* and *P. ostreatus* by 36.41 and 68.81 per cent respectively.

Hybridization was carried out between the compatible single spore cultures of *P. djamor* with *P. florida* and *P. ostreatus* independently by dual culture method. The hybrids were confirmed by the presence of clamp connection in the mycelium from the interaction zone. The BE of the hybrids *viz.*, *P. djamor* x *P. florida* and *P. djamor* x *P. ostreatus*, was improved by 34.40 and 48.49 per cent respectively over the parent, *P. djamor*. The days taken for primordial initiation in the hybrids were significantly reduced compared to their parents. The days were reduced in hybrid, *P. djamor* x *P. florida* by two and 13 days respectively than the parents; and three and 12 days in hybrid of *P. djamor* x *P. ostreatus*, compared to its respective parents. The hybrids showed blended characters of both their parents. Light pink coloured sporocarps with wavy margin and stout stipe was produced by the hybrid, *P. djamor* x *P. florida* and light pink coloured sporocarps with slight depression towards the base and increased stipe length was produced in the hybrid, *P. djamor* x *P. ostreatus*.

Studies on the effect of gamma irradiation at 20 and 25 Gy in strain improvement revealed that BE of *P. djamor* increased by 9.25 per cent when irradiated at 20 Gy. The number of days taken for primordial initiation was also reduced by one day compared to *P. djamor*. Though *P. florida* irradiated at 25 Gy recorded increase in sporocarp size, there was no significant difference in the BE over *P. florida*. The BE of *P. ostreatus* irradiated at 25 Gy was improved by 12.89 per cent and there was earliness in primordial initiation by three days compared to the parent, *P. ostreatus*.

The improved cultures were evaluated for their relative performance and it was found that BE was higher for the mutant of *P. ostreatus* irradiated at 25 Gy and increased by 12.3 per cent over the parent. Whereas the hybrids which recorded earliness in primordial formation with pink coloured slightly delicate sporocarps and improved BE were also found to be promising.

Molecular characterisation of improved strains along with parents were analysed by RAPD (Random Amplified Polymorphic DNA). Pairwise comparisons of the strains, based on the presence or absence of unique and shared amplicons, were used to generate similarity coefficient of Jaccard. The results were then analysed using the unweighted pair-group method with arithmetic average (UPGMA). The analysis revealed that the hybrid (*P. djamor* x *P. ostreatus*) had 57.1 per cent similarity with *P. djamor* and 28.6 per cent similarity with *P. ostreatus*. The hybrid, *P. djamor* x *P. florida* had 66.7 and 50 per cent similarity with *P. djamor* and *P. florida* respectively. The mutant strain of *P. ostreatus* at 25 Gy recorded 22 per cent polymorphism with the control.

The present study indicated the exploitability of two promising strains. The hybrid of *P. djamor* and *P. ostreatus* can be recommended for the production of slightly delicate, light pink sporocarps with reduced crop period (44.75 days). Whereas *P. ostreatus* irradiated at 25 Gy produced delicate white sporocarps with increased BE and reduced crop period by one week compared to the parent.