CONSERVATION AND CHARACTERIZATION OF *PLEUROTUS* VARIABILITY OF INDIA

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ABSTRACT

The present investigation was undertaken to conserve, characterize, domesticate and commercialize the *Pleurotus* variability prevalent in different geographical regions of India. The species /isolates varied greatly in their cultural, nutritional, morphological and agronomical characters. All the species/isolates were tropical in nature requiring 25-30 °C for both vegetative growth and sporophore induction. Among the five species/isolates studied, the Madurai isolate showed molecular resemblance to *P. floribundus*, the isolate from Western Ghats showed resemblance to *P. djamor* var. *roseus*, pink isolate from Balaghat forest region of Madhya Pradesh belonged to *P. djamor*, the white isolate from the same region also showed 70% resemblance to *P. djamor* group. The Balaghat isolates showed very high contents of iron and zinc content and hence can play an important role in mitigation of malnutrition in India.

Keywords: conservation, India, *Pleurotus*, variability

INTRODUCTION

Pleurotus species commonly called as 'oyster mushrooms' are one of the most gregarious of the cultivated mushrooms with wide adaptability in terms of environment and growing substrate. India, due to its varied geography and forest types is rich in mushroom diversity which awaits its conservation and domestication. The present investigation was undertaken to conserve and characterize the variability of *Pleurotus* species found in India for a better scope of diversification of the Indian mushroom industry.

MATERIALS AND METHODS

Explorations

Explorations were undertaken in the western Ghats of Karnataka (Shimoga district) lying between the latitudes 13°27' and 14°39' N and between the longitudes 74°38' and 76°04' E at a mean altitude of 640 meters above sea level. Shimoga district is a part of the Malnad region of Karnataka and is also known as the 'Gateway to Malnad' or 'MalenaadaHebbagilu' in Kannada. A pink *Pleurotus djamor* was collected from this region. The second exploration was undertaken in the forest of Madhya Pradesh (Balaghat district) located at 21°48' N 80°11' E. It has an average elevation of 288 meters. A pink and a white *Pleurotus* spp./isolates were collected from these forests. The third exploration was done in the Hessaraghatta campus of IIHR in Bangalore district lying in the southeast of Karnataka. It is positioned at 12.97° N 77.56° E at an average elevation of 920 meters. Bangalore is located in the heart of the Mysore Plateau (a region of the larger Deccan Plateau). The blackish gray *P. cystidiosus* was collected growing on Singapur cherry tree (*Muntingia calabura)* from this area. The fourth exploration was undertaken in the forest region of Madurai circle in Tamil Nadu. Madurai is located between latitude: 9°55' 59" N and longitude: 78°07' 00" E at an elevation of 136 meters. A white *Pleurotus* spp/isolate was collected from this region.

Pure culture

Tissue culture of the species/isolates were raised from sporophores collected from wild on malt extract agar medium (MEA) comprising of 50 g malt extract agar powder (Himedia Pvt. Ltd. Mumbai) dissolved in 1000 ml of distilled water,

sterilized at 121 °C, 15 psi pressure for 20 minutes in glass culture tubes. The cultures were purified by sub culturing in Petri plates (90 mm diameter) on the same medium. The pure cultures were stored in refrigerator for further studies.

Validation studies

Mother spawn was made on half boiled sorghum grains in 500 ml glass bottles by the traditional grain spawn method and incubated at 27±2 °C. The fully colonized mother spawn was used for further inoculation of planting spawn. The substrate for planting spawn was similar as mother spawn but the container used was 800mm x 1200mm, 150 gauze polypropylene (PP) bags and plugged with non absorbent cotton. Spawn substrate preparation, sterilization and incubation were done as described earlier. After complete colonization, the planting spawn was used for sporophore induction studies.

Sporophore induction was studied on sterilized (121°C, 15 psi pressure for 15 minutes) paddy straw having 65±2% moisture filled in polypropylene (PP) bags (1000mm x 1200mm, 150 gauze thickness). Each bag contained 1000 g of wet paddy straw and inoculated with 50 g of planting grain spawn. The bags were incubated at 26±2 °C for spawn running after which they were shifted to cropping rooms and pierced with 25 mm diameter holes (4 holes per bag). Temperature of 26±2°C, humidity of 80-85% and light (two 40 w fluorescent tubes for 8 hour per day) was maintained in the cropping rooms. Ventilation was through 4 mesh (insect proof) open ventilators (590 x 450mm) in each cropping room. The sporophores of the first flush were harvested and fresh tissue cultures raised and conserved as described earlier.

Cultural characterization

Cultural characteristics were investigated by evaluating various physiological factors (media, temperature and pH) required for optimal vegetative growth and conservation. Media tested for optimal culture growth included: malt extract agar (MEA), potato dextrose agar (PDA), potato malt agar (PMA), potato carrot agar (PCA) and oatmeal agar (OMA), ready-to-use dehydrated powder from Himedia Laboratories Pvt Ltd, Vadhani Industrial Estate, LBS Marg, Mumbai, India) and Raper's Complete medium (RC) containing 20.0 g glucose, 2.0 g peptone, 2.0 g yeast extract, 0.50 g magnesium sulphate, 0.46 g potassium dihydrogen phosphate and 20.0 g agar powder per liter of distilled water. Wheat extract agar (WEA) and rice bran agar (RBA) was prepared by boiling 30.0 g of whole wheat grain or rice bran powder in 500 ml of distilled water for one hour, followed by decanting and filtering through several thicknesses of cheese cloth padded with cotton. The final volume was made up to one liter with distilled water and 20.0 g agar powder was added. All the media were sterilized in autoclave at 15 lb pressure for 20 minutes. The effect of temperature on mycelial growth was conducted on MEA. Plates of MEA media were inoculated with 7 mm mycelial discs taken from the periphery of 10 days old pure culture. Plates were incubated at variable temperatures (15-40 °C at intervals of 5 °C) and the radial growth was recorded at an interval of 2 days. The effect of pH was studied in malt extract broth (malt extract powder 30.0 g, peptone 5.0 g per litre of distilled water) of variable pH (3.0-8.0, at intervals of 0.5 pH) and the mycelial dry weight was recorded as the measure of mycelial growth. Microscopic studies of the vegetative mycelium and basidiospores were also undertaken.

Agronomical characterization

Fruiting trials for agronomical characterization were conducted as described under validation trials. Monthly crop evaluation was done for three years.

Nutritional characterization

The nutritional analysis included estimation of protein, fat, carbohydrate, vitamin B and minerals. The proximate nutritional composition was determined by the methods of AOAC [1]. Proximate analysis studies included the determination of crude fat (A.O.A.C. 7.056, 1980), crude fiber (A.O.A.C. 7.061, 1980) and crude protein (A.O.A.C.47.021, 1980) using the conversion factor (N X 6.25). Carbohydrate was determined by phenol-sulphuric method of Dubois [2]. Mineral nutrient analysis was done with oven dried (70p C) samples as per the method described by Piper [3] using KjeltekAut-Analyzer, Gerhardt, Germany and atomic absorption spectrophotometer (AAS). Water soluble vitamins biotin (B7), niacin (B3), pyridoxine (B6), pantothenic acid (B5), folic acid (B9), cyanocobalamine (B12), thiamine (B1) and riboflavin (B2) were analyzed by UPLC-MS/MS as per methodology given by Esteve *et al*.[4]

RESULTS AND DISCUSSION

Tables1 (a-c) represents the observations on cultural characterization of different *Pleurotus* species/isolates collected from different geographical regions. All the species/isolates could grow in a temperature range of 15-35 °C except *P. cystidiosus*, which showed mere initiation of growth at 15 °C. The optimum temperature for mycelial growth of all the species/isolates was 25-30 °C. The upper limit of temperature tolerance was 40 °C except for *P. cystidiosus*, which could not be revived after exposure to this temperature. The other species/isolates could be revived when brought back to optimal temperature after high temperature (40 °C) exposure for 12 days (Table 1a).

Table 1a. Effect of temperature on mycelia growth of wild *Pleurotus* isolates/species

Temperature (°C)	**Radial growth in mm/day**				
	***Pleurotus djamor* (Shimoga isolate)**	**Pink *Pleurotus* spp. (Balaghat isolate)**	**White *Pleurotuss* pp. (Balaghat isolate)**	***P. cystidiosus* (Bangalore isolate)**	**White *Pleurotus* (Madurai isolate)**
15	5.54	2.46	1.82	Only initiation	7.39
20	8.03	9.00	8.18	4.34	10.54
25	12.85	15.0	15.0	6.84	12.70
30	11.07	15.0	14.91	6.25	14.26
35	7.69	7.68	3.21	1.57	10.97
40	No growth	0.00	0.00	No growth (lethal)	0.00
CD at 1%	0.90	0.86	0.71	0.22	0.35

Table 1b. Effect of pH on myceial growth of wild *Pleurotus* isolates/species

pH	**Mycelial dry weight (mg)**				
	***Pleurotus djamor* (Shimoga isolate)**	**Pink *Pleurotus* spp. (Balaghat isolate)**	**White *Pleurotus* spp. (Balaghat isolate)**	***P. cystidiosus* (Bangalore isolate)**	**White *Pleurotus* (Madurai isolate)**
3	No growth	No growth	No growth	5.2	0.00
3.5	73.96	67.2	88.4	6.02	0.00
4	66.1	42.2	81.6	5.2	52.34
4.5	61.23	102.4	130.2	91.8	67.40
5	146.26	157.6	108.8	102.14	103.62
5.5	237.40	229.8	127.4	331.58	132.08
6	202.2	184.6	151.4	348.34	139.94
6.5	275.43	220.4	208.2	436.9	143.80
7	250.23	258.2	232.2	382.36	147.28
7.5	272.2	132.6	233.6	381.3	138.98
8	250.63	289	250	364.38	134.36
8.5	234.9	164.2	220.8	357.06	133.86
9	285.3	98.4	234.6	350.2	130.70
CD at 1%	46.5342	150.72	84.86	57.45	4.15

Table 1c. Effect of different media on mycelia growth of wild *Pleurotus* isolates/species

Media	Mycelial dry weight (mg)				
	***Pleurotus djamor* (Shimoga isolate)**	**Pink *Pleurotus* spp. (Balaghat isolate)**	**White *Pleurotus* spp. (Balaghat isolate)**	***P. cystidiosus* (Bangalore isolate)**	**White *Pleurotus* (Madurai isolate)**
MEA	12.85	18.0	18.0	3.94	12.79
RC	12.21	18.0	18.0	4.61	12.48
PDA	11.25	17.0	18.0	3.74	12.5
OMA	9.21	14.33	12.33	3.21	7.76
WEA	9.4	Not studied	Not studied	4.91	8.79
RBA	10.75	Not studied	Not studied	4.39	12.33
PMA	12.08	17.86	18.0	2.91	8.33
PCA	10.41	18.0	18.0	3.75	7.08
CD at 1%	0.99	1.89	1.21	0.48	0.29

MEA=Malt extract agar, RC= Rapers complete medium, OMA=Oat meal agar
WEA=Wheat extract agar RBA=Rice bran agar, PDA=Potato dextrose agar
PMA = Potato malt agar, PCA = Potato carrot agar

The pH tolerance of *P. djamor* (Shimoga isolate), pink and white *Pleurotus* spp. (Balaghat isolates) was 3.5-9.0 optimum being 5.5-9.0 (Shimoga isolate), 5.5 – 7.0 (pink *Pleurotus* spp, balaghat isolate), 6.5 – 9.0 (white *Pleurotus* spp, balaghat isolate), 5.5 – 9.0 (*P. cystidiosus*) and 5.5 – 9.0 (white *Pleurotus* spp, Madurai isolate). All the species/isolates could grow on the commonly used mycological media like MEA, PDA and RC medium. However, *P. cystidiosus* showed better growth on WEA.

Phenotypic variability among the species/isolates has been shown in Table 2. The sporophore color was pink (Shimoga & Balaghat isolates), white (Balaghat & Madurai isolates) and black *(P. cystidiosus* from Bangalore). The sporophore color gradually became lighter on maturity. The pileus margin varied from smooth to lobed. Stipe varied from rudimentary to well formed cylindrical among the isolates. Position of the stipe varied from eccentric to sub eccentric to central.The agronomical variability has been shown in table 3. All species/isolates could be grown on sterilized/pasteurized paddy straw. *P. djamor* (Shimoga isolate) and pink *Pleurotus* spp. (Balaghat isolate) were short duration crops with a cropping cycle (period from spawning to getting 80% harvest) of 18-32 days. White *Pleurotus* spp. (Balaghat isolate) required 25-62 days, white *Pleurotus* spp. (Madurai isolate) required 35-48 days and *P. cystidiosus* (Bangalore isolate) required 35-48 days. *Pleurotus djamor* (Shimoga isolate), white *Pleurotus* spp. (Madurai isolate) and white *Pleurotus* spp. (Balaghat isolate) gave higher sporophore yield compared to pink *Pleurotus* spp. (Balaghat isolate) and *P. cystidiosus* (Bangalore isolate). The post harvest shelf life of *P. cystidiosus* (Bangalore isolate) was superior than other isolates. It could be stored for 3-4 days at room temperature (27-29 °C) and for 15-30 days at 4-5 °C depending on maturity at the time of harvest. The other species/isolates showed poor to moderate shelf life (Table 4).

Tables 5 a & b show the nutritional status of different species/isolates. Highest crude protein was observed in white *Pleurotus* (Madurai isolate), followed by pink *Pleurotus* isolates (Balaghat & Shimoga isolates). Highest carbohydrate content was in pink *Pleurotus* (Shimoga isolate) and lowest in *P. cystidiosus* (Bangalore isolate). The highest crude fiber content was in *P. cystidiosus* (Bangalore isolate). Among the minerals both white and pink *Pleurotus* (Balaghat isolates) and white *Pleurotus* (Madurai isolate) showed highest iron content. Highest zinc content was observed in Balaghat isolates followed by Shimoga isolate. In general all the isolates were low on calcium and high on potassium (Table 5a). Highest biotin content was found in pink *Pleurotus* spp. (Balaghat isolate) followed by white *Pleurotus* spp from the same region and *P. cystidiosus* (Bangalore isolate), highest niacin was in white *Pleurotus* spp. (Balaghat isolate) followed by *P. cystidiosus* (Bangalore isolate). Pyridoxine was higher in pink *Pleurotus* spp. (Balaghat isolate) followed by *P. djamor* (Shimoga

Table 2. Phenotypic variability among wild *Pleurotus* isolates/species

Phenotypic characters	***Pleurotus djamor* (Shimoga isolate)**	**Pink *Pleurotus* spp. (Balaghat isolate)**	**White *Pleurotus* spp. (Balaghat isolate)**	***P. cystidiosus* (Bangalore isolate)**	**White *Pleurotus* (Madurai isolate)**
Color of pileus at pinhead stage	Pink	Pink	White	Black	White
Color of pileus at mature stage	Pink (lighter as compared to pinhead stage)	Pink (lighter as compared to pinhead stage)	White	Black, sometimes fading to brown	White
Shape of pileus	Pleurotoid	Pleurotoid like flower due to central stipe	Pleurotoid becoming funnel shaped on maturity	Pleurotoid like on maturity	Pleurotoid becoming flattened plate
Margin of pileus	Smooth	Lobed becoming like a flower	Wavy	Smooth	Smooth
Pileus height (point of attachment to stipe to the edge of pileus) (mm)	Range - 30.19-66.34 Average - 49.9	Range – 35.27 Average – 26.95-52.70	Range – 25.8 – 38.5 Average – 31.5	Range -56.84 - 130.5 Average – 73.92	Range 8.6 – 23.5 Average -15.46
Pileus width (from one edge of the pileus to the other edge)(mm)	Range- 25.18-40.19 Average - 33.94	Range -19.50-56.32 Average-32.21	Range – 27.5 – 62.7 Average – 37.22	Range -72.07-100.5 Average – 82.60	Range -17.7 – 31.6 Average – 25.26
Color and texture of stipe at pinhead stage	Pink, tough	Whitish pink	White	Gray brown, hard	White
Color of stipe at harvest stage	Same as above	Same as above	Same as above	Same as above	Same as above
Position of stipe	lateral	Eccentric to central	Eccentric to central	Lateral	Eccentric to central
Stipe length (mm)	Range-12.79-39.61 Average - 25.40	Range- 24.79 – 47.47 Average - 32.47	Range – 33 - 57 Average – 41.79	Range – 33.05-84 Average – 45.92	Range – 8.6 - 34.35 Average -25.75
Stipe thickness (mm)	Range - 3.21-7.29 Average - 5.56	Range -5.86 – 11.37 Average - 8.75	Range – 8.66 - 12 Average – 10.33	Range –9.05 – 16.2 Average – 11.90	Range– 3.83 – 6.23 Average -4.63
Color of gills	Pink	Pink	White	Creamish white	White
Coremia	No	No	No	Yes, black heads with white stalks, single celled	No
Color of spore print	White with some pink shade	White with some pink shade	White	White with grayish tinge	White
Microscopic features	Hyphae dimitic, basidiospore cylindrical (18.50 x 11.73 µ) with numerous sterile spores (22.01x 12.46 µ)	Hyphae dimitic, basidiospore cylindrical (19.48 x 10.94 µ)	Hyphae dimitic, basidiospore cylindrical (19.56 x 9.79 µ)	Hyphae monomitic, conidia one celled, basidiospores cylindrical, (11-14 x 4-5µ)	Hyphae dimitic, basidiospore cylindrical (23.41 x 11.20 µ)

Table 3. Agronomical characters of wild *Pleurotus* isolates/species

Agronomical parameters	*Pleurotus djamor* (Shimoga isolate)	Pink *Pleurotus* spp. (Balaghat isolate)	White *Pleurotus* spp. (Balaghat isolate)	*P. cystidiosus* (Bangalore isolate)	White *Pleurotus* (Madurai isolate)
Spawn running period (days)	18-19	18-19	23-24	24-32	20-22
Time taken for 1st harvest after opening of bags (days)	6-7	6-7	9-10	13-27	19-20
Average yield of 1st flush (g per kg wet substrate)	106.37	74.38	117.51	42.10	120.68
Average yield of 2nd flush (g per kg wet substrate)	33.62	26.00	48.15	59.76	55.16
Average yield of 3rd flush (g per kg wet substrate)	32.70	16.82	51.33	44.73	46.66
Period to obtaining 80% or more of total yield after opening of bags (days)	4-10	4-10	7-33	19-60	15-31
Cropping period from spawning to getting 80% or more of total yield (days)	18-32	19-30	25-62	42-90	35-48
Cropping cycle from spawning to cleaning of rooms (days), 2 cleaning days common for all	20-34	21-32	27-64	44-92	37-50
Number of possible crops in 365 days in a given space	18.25-10.73	17.38-11.40	13.51-5.70	8.29-3.96	9.86-7.3

Table 4. Post harvest storage studies of wild *Pleurotus* isolates/species

Species/isolate	Post harvest storage in trays wrapped with antifog film (27-29 °C)	Post harvest storage in trays wrapped with antifog film (4-6 °C)
Pleurotus djamor (Shimoga isolate)	Shelf life 1-2 days depending on the harvesting stage, sporophore color fades faster on storage. No aerial hyphae visible on sporophores even after 48 hours, not soggy	Shelf life 4-6 days depending on the harvesting stage, color fades slowly on storage, No aerial hyphae visible on sporophores, not soggy
Pink *Pleurotus* spp. (Balaghat isolate)	Similar to pink isolate from Western ghats	Similar to pink isolate from Western ghats
White *Pleurotus* spp. (Balaghat isolate)	Same as for pink isolate from the same region	Same as for pink isolate from the same region
P. cystidiosus (Bangalore isolate)	Shelf life 3-4 days depending on the harvesting stage, color fades on storage. No aerial hyphae visible on sporophores even after 3-4 days. The black sporophores show better shelf life than lighter colored sporophores, not soggy	Shelf life 20-30 days depending on sporophore maturity, no odor or softening. No aerial hyphae visible on sporophores even after 25-30 days storage at low temperature, not soggy
White *Pleurotus* (Madurai isolate)	Shelf life 1-1½ days, browning starts after 24 hours, aerial growth visible on stipe by 36 hours, becoming soggy	Shelf life 3-5 days, aerial growth visible on stipe by 5th day, becoming soggy

Table 5a. Proximate composition and mineral content of wild *Pleurotus*species/isolates

Nutritional parameters	*Pleurotus djamor* (Shimoga isolate)	Pink *Pleurotus* spp. (Balaghat isolate)	White *Pleurotus* spp. (Balaghat isolate)	*P. cystidiosus* (Bangalore isolate)	White *Pleurotus* (Madurai isolate)
Crude Protein (% dw)	29.81	30.87	24.56	24.68	36.25
Carbohydrates (% dw)	50.59	51.41	53.22	33.43	UI
Fat(% dw)	4.5	5.1	4.9	5.04	UI
Crude fiber(%) dry weight basis	13.2	15.9	14.2	27.80	UI
Nitrogen (%) dry weight basis	4.77	4.94	3.93	3.95	5.8
Calcium (%) dry weight basis	0.001	0.12	0.005	0.21	0.002
Potassium (%) dry weight basis	2.23	1.66	1.71	2.1	2.87
Phosphorus (%) dry weight basis	1.09	0.60	0.60	0.55	1.49
Magnesium (%) dry weight basis	0.09	0.102	0.085	0.12	0.11
Iron (µg/g) dry weight basis	192	350	284	59	232
Zinc (µg/g) dry weight basis	156.56	201	169	111	130

UI = under investigation

isolate), white *Pleurotus* spp. (Balaghat isolate) and *P. cystidiosus* (Bangalore isolate). Pantothenic acid was highest in white *Pleurotus spp.* (Balaghat isolate) followed by pink *Pleurotus* spp. (Balaghat isolate), *P. cystidiosus* (Bangalore isolate) and *P. djamor* (Shimoga isolate). Folic acid was highest in the white and pink *Pleurotus* spp (Balaghat isolates) followed by *P. djamor* (Shimoga isolate) and *P. cystidiosus* (Bangalore isolate). Cyanocobalamine was highest in *P. cystidiosus* (Bangalore isolate) followed by white and pink *Pleurotus* spp (Balaghat isolates) and *P. djamor* (Shimoga isolate). Thiamin was highest in *P. cystidiosus* (Bangalore isolate) and in white *Pleurotus spp.* (Balaghat isolate) followed by pink *Pleurotus* spp. (Balaghat isolate) and *P. djamor* (Shimoga isolate). Riboflavin was highest in pink and white *Pleurotus* spp (Balaghat isolates) followed by other isolates/spp (table 5b).

Table 5b. Water soluble vitamins of wild *Pleurotus* species/isolates

Vitamin	*Pleurotus djamor* (Shimoga isolate)		Pink *Pleurotus* spp. (Balaghat isolate)		White *Pleurotus* spp. (Balaghat isolate)		*P. cystidiosus* (Bangalore isolate)		White *Pleurotus* (Madurai isolate)
	FW	DW	FW	DW	FW	DW	FW	DW	
Biotin (B7) mg/100g	0.236	1.269	0.324	2.160	0.274	1.724	0.198	1.081	UI
Niacin (B3) mg/100g	1.968	10.585	1.321	8.807	3.659	23.021	3.216	17.551	UI
Pyridoxin (B6) mg/100g	0.102	0.549	0.124	0.827	0.094	0.591	0.071	0.387	UI
Pantothenic acid (B5) mg/100g	0.949	5.104	1.159	7.727	1.959	12.325	1.312	7.160	UI
Folic acid (B9) µg/100 gm	3.200	17.212	4.800	32.000	5.200	32.717	2.100	11.460	UI
Cyanacobalamine (B12) µg/100 gm	0.761	4.093	0.954	6.362	1.742	10.962	2.612	14.257	UI
Thiamine (B1) mg/100g	0.106	5.753	0.103	6.879	0.117	7.402	0.139	7.594	UI
Riboflavin (B2) mg/100g	0.226	1.216	0.654	3.693	0.286	1.799	0.214	1.168	UI

FW = Fresh weight DW = Dry weight UI = under investigation

Numerous studies in India have been conducted earlier on the occurrence of indigenous *Pleurotus* species [5, 7]. However, none of these studies were focused on end to end study from conservation to domestication and commercialization. This is the first attempt to present a complete investigation on *Pleurotus* variability of India with a view to conserve and develop a complete technology for the utilization of the wild species. Similar studies were conducted by Lechner *et al*. in Argentina [8]. The authors reported the occurrence of three varieties of *P. djamor*: var *djamor*, var. *cyathiformis* and var. *roseus* and *P. cystidiosus*. These two species seem to be widely distributed in the tropical and subtropical regions. The wild species were very high in Vitamin B, protein, iron and zinc content. Hence these wild species can play a pivotal role through fortification in mitigating mineral malnutrition and for breeding nutritionally rich commercial strains suitable for India.

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