of a cell wall and osmotic sensitivity were the criteria used in defining protoplasts. Good protoplasts yields were obtained with novozyme + cellulase combination but pretreatment with dithiothreitol (DTT) was necessary to achieve this; thermomycolase (extra and intracellular) of *Malbranchea* produced over 65% protoplasts under these experimental conditions. A combination of extra- and intra-cellular thermomycolase was able to release 70-80% protoplasts from DTT-treated *S. cerevisiae* cells. The generality of this result is supported by the work of Thomas *et al*[7] and Masilkova *et al*[8]. In other combinations described in table 1, novozyme was no better than *Malbrancea* lytic preparation. However, a mixture of extra- and intracellular thermomycolase with novozyme 234 proved most effective as it released 90–95% protoplast from *S. cerevisiae* within 2–3 hr. As one would note all the four strains of the yeast were equally sensitive to mycolytic preparation from *Malbranchea*.

The mycolytic preparation of *Malbranchea* was also noteworthy with regard to the rate of release of protoplasts (figure 1). Thus, a mixture containing extra- and intracellular mycolase plus novozyme 234 released 60–70% protoplasts within 60–120 min; nearly 95% of the cells released protoplasts after 3 hr. In contrast, only 50–60% cells were converted to protoplasts in a similar preparation but which lacked novozyme. However, as evident from the data in table 1, part of this was overcome by the addition of DTT.

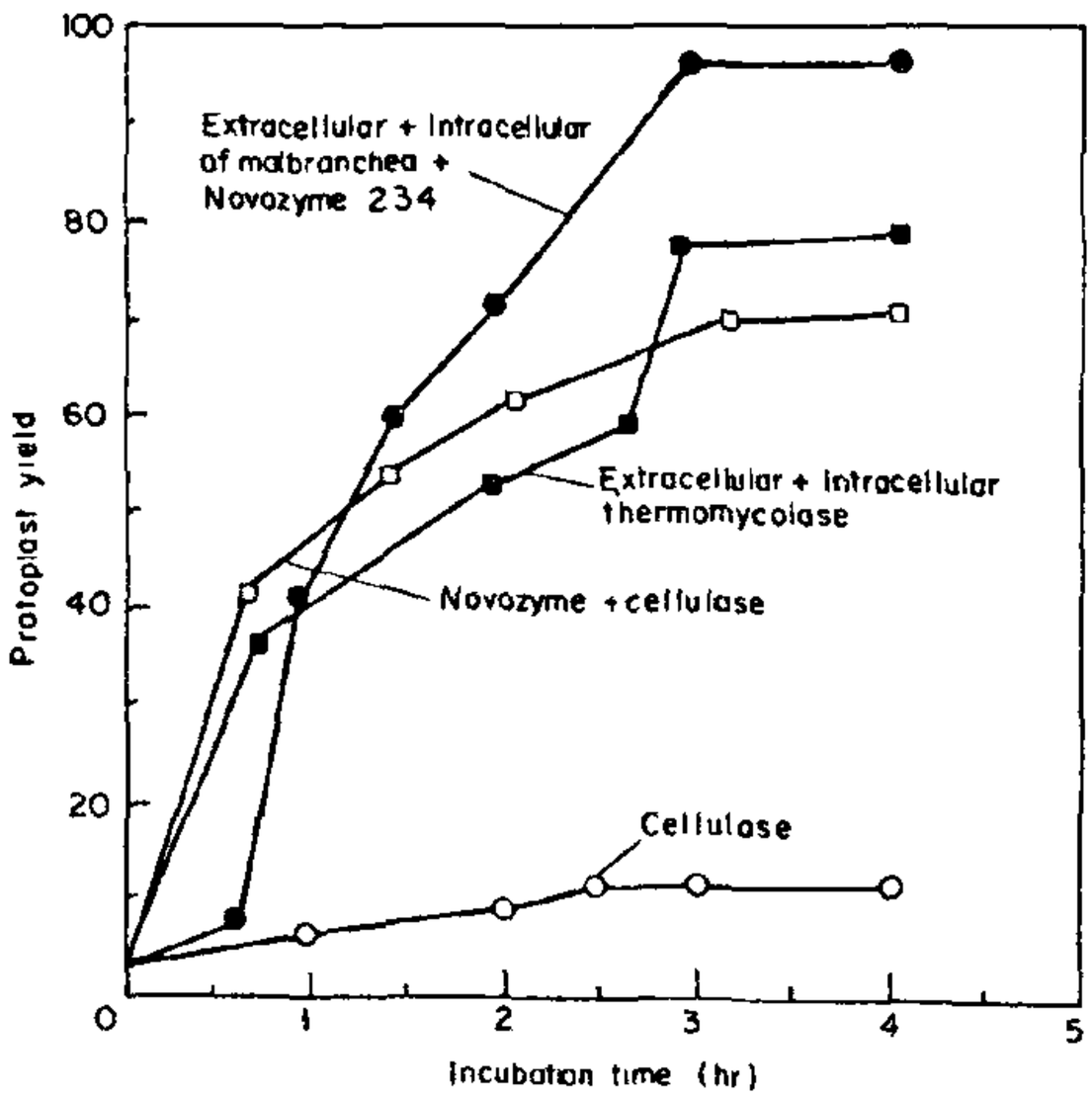

Figure 1. Effect of enzyme combination with incubation time on protoplast release.

The enzyme preparation from the thermophile exhibited considerable proteolytic activity but protoplasts suspended in this solution did not burst even after holding them for 36 hr. Also, protoplasts prepared using the thermomycolase of *Malbranchea* were viable and regenerated to form colonies on agar. In general high level of β-D-Glucanase and chitinase activities were associated with the enzyme that gave 64% protoplast yield (*i.e.* Novozyme 234 and cellulase (Merck)). The importance of these activities in the digestion of fungal cell walls has been reported earlier[9].

Efforts are in progress to separate and characterize some of the more important enzymes involved in the digestion of yeast cell walls with a view to develop a suitable methodology for commercial exploitation. No commercial enzyme is currently manufactured from *Malbranchea* but results of this investigation appear very promising to undertake large scale production of thermomycolase.

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RHIZOSPHERE SOIL NITROGENASE (C_2H_2 REDUCTION) AS INFLUENCED BY RICE VARIETY

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RECENT studies from the temperate and sub-tropic regions of the rice growing countries indicate wide

differences in the *in situ* rhizosphere N_2 fixation between rice varieties[1–3].

A field experiment was conducted at CRRI, Cuttack to evaluate the varietal variation in rice with regard to the nitrogenase activity during 1984 in the Institute farm. Seedlings (15–20 day old) of 20 high yielding rice varieties were transplanted under uniform field conditions in three replicate micro-plots (3 m^2) under submerged conditions. Recommended dose of fertilizer (30 kg N/ha) as urea was applied as a basal dressing. Rhizosphere soil was collected (3 samples from three plants from each plot) at different intervals. Rhizosphere soil (2 g fresh weight) was transferred to 125 × 16 mm B-D vacutainer tubes for C_2H_2 reduction analysis. The incubation and nitrogenase were analysed following the methods described earlier[4–6] on a gas chromatograph fitted with hydrogen flame ionization detector.

Nitrogenase activity varied with the growth phase of the plant and among the rice cultivars tested (table 1). Results further indicate that the majority of the varieties had high activity after 73 days of transplanting which declined, at least in a few varieties, after 112 days. However, variations, but not consistent, were also observed among and within the rice variety. Reports indicate that nitrogenase varied both with the plant species and the soil type[7]. The variation observed in the nitrogenase in the rhizosphere soil samples in the present study could not be attributed to the habitat or soil type since all the varieties were grown under uniform field conditions on the same soil and the rhizosphere samples were collected at the same time from all the varieties.

The varieties used in this study varied in their duration and perhaps this could be one factor responsible for variation in nitrogenase. Lee *et al*[8] observed high correlation between the root weight of various varieties and nitrogen fixation. Presumably, the difference in the nature of the organic material supplied by the root might also be responsible for the alterations in the nitrogenase in the rhizosphere. Moreover, the variation among the rice cultivars suggests the degree of association of N_2 fixers[1,3].

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Table 1. *Rhizosphere soil nitrogenase activity as influenced by different rice varieties*

	Nitrogenase activity (n moles of C_2H_4 formed/g soil/day)					
	Days after transplanting					
Rice variety	67	73	92	103	112	120
Pallavi	86	110	334	423	211	293
Kalinga-I	120	233	480	421	307	163
Ratna	143	123	300	251	397	102
Pusa 2–21	196	100	299	208	419	900
Annapurna	243	97	500	448	437	616
Bala	298	174	413	748	400	350
Kalinga-II	127	191	872	546	343	506
Shakti	60	167	695	722	525	751
Jaya	353	132	764	752	444	749
Jagannath	298	159	734	725	422	580
Pankaj	105	94	701	741	341	512
IR 8	280	107	405	694	557	338
TN-1	201	193	381	700	408	416
Padma	227	207	410	586	429	607
Vani	413	118	717	788	441	464
Sona	376	252	361	924	593	246
Supriya	368	211	485	690	609	508
Kalinga-III	465	200	867	703	843	395
Sattari	408	222	386	551	485	449
Madhu	345	212	234	810	628	299
LSD 5%	87	89	160	162	172	99
1%	117	119	214	217	231	133

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Table 1 *Morphometeric differences between female and male fish.*

	Female	Male
Standard length (mm) (length)*	19.7 ± 0.6	19.2 ± 0.7
Longest dorsal ray	15.2	20.3
Longest ventral ray (number)	11.7	11.7
Anal fin base	30.1	31.9
Anal fin rays branched	0.92	0.17
Rays with papilla processes	0	0
Nodes in an anal fin ray	8.9	13.1
Nodes in a dorsal fin ray	8.6	12.5
Urinogenital papillae	Large	Small

* Expressed as percentage of the standard length.

SEXUAL DIMORPHISM IN *ORYZIAS MELASTIGMA* (McCLELLAND).

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ORYZIAS MELASTIGMA belongs to the order Atherinoformes, family Oryziatidae. The members of the genus *Oryzias* are small, hardy and prolific breeders which inhabit fresh and brackish water. They are worldwide in distribution (tropical and temperate regions). Yamamoto[1] and Labhart[2] reported 10 species of *Oryzias*, of which *O. melastigma* is the only representative from India not noticed by the past investigators due to its restricted distribution. The information available on *Oryzias melastigma* is only the work of Sriramulu[3] and Uwa *et al*[4].

Morphometric differences in male and female *O. melastigma* are given in table 1. Sexual dimorphism is exhibited in figures 1 and 2.

From table 1 it is evident that sexual dimorphism is quite conspicuous in this fish. Although the females and males are almost of the same size, yet the anal and dorsal fins are enlarged in the males while the urinogenital papilla in front of the urinogenital pore is comparatively small. On the contrary, the females have less developed anal and dorsal fins but well developed urinogenital papilla. Males very active in mating behaviour reveal the black ventral fins (figure 1). The most prominent feature of the male

Figure 1. *Oryzias melastigma* (approximately × 2) **A.** Female **B.** Male

sexual character is the shape of the anal fin which is almost triangular while in female it is almost like a parallelogram. The branched anal fin rays are less in males than in females. The nodes on anal fin ray and dorsal fin ray are more in males than females. The eggs remain hanging under the bellies of females for several hours (figure 2) after spawning. The eggs are kept in