

# RICE RESEARCH STATION, VYTTILA

(1958 - 2013)

# FIVE DECADES OF GLORIOUS RESEARCH

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FIVE DECADES OF GLORIOUS RESEARCH AT THE RICE RESEARCH STATION, VYTTILA

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# Foreword

Preface

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# Foreword

Rice is the most important crop in India which plays a vital role in food security. Approximately 90% of the rice is being produced and consumed in tropical Asia. India stands first in the world in rice area (44.3 m ha) and second in production (93.4 m t) while China tops in production and comes second in area.

Rice farming has an annual value of over 150 billion US dollars and directly or indirectly affects over 2 billion people who either depend on rice as their staple food or are involved in its production. Rice is often the only food that 400 million of the chronically hungry have to eat.

The major problems related to rice production and productivity in India are the dwindling area under cultivation due to urbanization and fragmentation of the land, non-availability of farm labourers in time, high cost of cultivation, reduced water availability for irrigation, deficiency of macro, secondary and micro nutrients, low nutrient use efficiency, toxicity due to adverse soil problems, biotic stresses like pests and diseases, abiotic stresses like salinity, acidity, submergence, inadequate storage facilities, poor marketing facilities, low support price and lower productivity under the changing climate and other natural calamities. Therefore the present need is to develop improved high yielding rice varieties suited to different agroecosystems and changing climate to develop appropriate crop production and plant protection technologies to combat the above mentioned problems.

I am happy to learn that the Rice Research Station, Vyttila has started research on saline farming of rice from 1958 onwards and could develop and contribute eight high yielding saline tolerant rice varieties to the farming community. These varieties have the capacity to produce and yield more than 4.0 t/ha without the addition of any chemical fertilizer or plant protection chemicals. The rice produced under this ecosystem is purely organic and has medicinal value owing to the high content of antioxidants like oryzanol, tocopherol and tocotrienols. This centre could also develop an organic protocol for rice production and suitable models for rice based integrated farming. I understand that the scientists worked in this renowned institute have made notable achievements in maintaining a good saline tolerant rice germplasm, varietal development and development of suitable integrated farming models.

Recently the centre has stepped forward in the field of biotechnology and started introgression of desired gene/multiple genes into the mega rice varieties through marker assisted backcrossing. A molecular breeding programme for introgression of salt tolerance (*Saltol QTL*) and submergence tolerance (*Sub 1* QTL) into three mega rice varieties of Kerala viz., Jyothi, Uma and Jaya is under way to mitigate the adverse effects of climatic change. Further, the centre could also develop efficient protocols for the mass multiplication of orchids and banana and started production and distribution of quality Tissue Culture plants of banana and orchids to the farmers.

I congratulate the scientists for their earnest efforts and team work in achieving these goals and the significant contributions made to the farming community of the state.

The initiative of the scientists to summarize the research outcome of the station in the form of a publication which is useful to the researchers and the farming community is well appreciated in this context. I wish all success to the team of scientists and hope that the centre will continue to serve the farmers of the state with new developments and achievements.



Prof. (Dr.) P. Rajendran Vice Chancellor Kerala Agricultural University Vellanikkara, Thrissur



Kerala is blessed with diverse soil and climatic conditions suitable for cultivation of a variety of crops in different parts of the state. The area under cultivation is decreasing drastically due to developmental changes, social problems, shortage of labourers, high cost of production etc. which results in the declining food production. The steady increase in population and a constant decrease in food production may lead to famine in the near future. The "climate change" will worsen the situation further. Hence a comprehensive approach to increase the productivity of food crops is necessary to enhance income of farmers. Although there is drastic reduction in rice area in the state from early seventies (8.75 lakh ha), the productivity has increased by 49.8%. The present area under rice cultivation in Kerala is only 2.08 lakh ha with a total production of 5.7 lakh tons which is far below our rice requirement.

The Rice Research Station, Vyttila, the only salinity research centre of Kerala Agricultural University, was established in the year 1958 and taken over by the Kerala Agricultural University in the year 1974. The main mandate of the station is to evolve high yielding saline tolerant rice varieties suitable for the pokkali/ coastal saline agro eco system and to develop suitable crop production and crop protection technologies.

This centre could evolve eight saline tolerant high yielding rice varieties. The latest varieties being semi tall, non-lodging and high yielding which could yield more than 4.0 t/ha grain yield without the addition of chemical fertilizers or plant protection chemicals. Though the area under cultivation has decreased drastically due to many reasons, the productivity could be doubled in the available area. This is a significant research achievement.

The centre has now started advanced research in molecular breeding - Marker assisted back crossing for the introgression of abiotic stress tolerant genes (*Saltol & Sub.1*) into the popular rice varieties of Kerala viz., Uma, Jyothi and Jaya. A full-fledged biotechnology research laboratory has been established in the station and could develop commercially viable protocols for the mass production of tissue culture plants of banana and orchids. A new tissue culture lab has been commissioned for the commercial production of tissue culture plants of banana and orchids utilizing the fund from the Department of Agriculture and Co-operation, GOI and ICAR.

It is worth mentioning that the centre could establish an excellent analytical laboratory with sophisticated instruments and analysis of soil, water and plant samples for both macro and micro nutrients is being carried out. The centre could render analytical service to other research centres of the University, Department of Agriculture and the farming community as well.

I congratulate the scientists and supporting staff worked in this station for their earnest effort in their research work to come out with successful results and to compile the research findings in the form of a publication. I wish all success to advance further in their future endeavours.



Dr.T.R.Gopalakrishnan Director of Research Kerala Agricultural University Vellanikkara, Thrissur

# Rice Research Station, Vyttila

# A brief history

The coastal belt of Kerala has a unique system of paddy cultivation in saline soils known locally as 'pokkali cultivation' or 'saline farming'. The term Pokkali refers to a saline resistant rice variety largely cultivated in Ernakulam District and it is synonymous with the Kaipad cultivation of Kannur district and Kariland cultivation of Alapuzha district. The salient characteristics of these fields are that the rice can be cultivated only during the low saline phase / monsoon season which starts from June-October. From November onwards salinity builds up and the high salinity in water makes the paddy cultivation impossible. The fields which lie fallow during this time are utilized for a traditional type of prawn and fish culture known as prawn filtration or chemeen kettu. The total area under saline soils of Kerala was 26,400 ha, the major part of which is situated in Ernakulam District. Vyttila situated in the centre of the Pokkali region with high fluctuations in salinity was found ideal for conducting research on the problems of saline farming. It was with this view that a Rice Research Station was started at Vyttila during 1958 and it is the pioneer and the only one of its kind in Kerala with a main mandate of evolving high yielding rice varieties suited for this area.

This station was started functioning during the year 1958 under the Department of Agriculture, Kerala, in a leased land in Kunnara near Vyttila. It was shifted later in the year 1963 to the present site in a newly acquired land of 11.375 acres. In 1973, an additional area of 10.15 acres was acquired. Recently 0.77 acres was also added to this making the total area to 22.30 acres ie.8.91 ha of which 4.25 ha comes under wet land .Utilization of the area for various research purposes is shown below

Sl. No.	· Purpose	Area (ha)
1	Rice (wet land)	4.2500
<b>2</b> .	Fisheries ( ponds)	3.0550
3	Dry land area	1.6082
4	Total 😚	8.9132

The station was taken over by the Kerala Agricultural University in 1974, and since 1976, a separate wing for the Fisheries research started functioning. A unit of the Project for Investigation of Coconut (root) wilt disease of Kerala was also implemented from April, 1981. In March 1982 the station was brought as a subcentre under the National Agricultural Research Project.

From the year 2008 onwards, the Station started research on biotechnology for the development of suitable varieties of ornamentals including orchids, anthurium, zyngonium etc. Further, the station has started the commercial production and distribution of quality tissue culture plants of banana and orchids. A new tissue culture laboratory has been commissioned recently having the capacity to produce about 10 lakh tissue culture plants per year. The strengthening of the analytical laboratory with modern facilities for the estimation of major, secondary and micro nutrients of plant, soil and water is another major development of the station. The research on bio-fertilizer and bio-control agents and the mass production and distribution of bio-control agents like *Pseudomonas fluorescence* is another activity of the station.

Recently the station has initiated research on molecular breeding for the introgression of abiotic stress tolerant QTLs into the mega rice varieties of Kerala. The introgression of salt tolerant QTL (*Saltol QTL*) from Pokkali and submergence tolerant QTL (*Sub 1QTL*) from FR -13 A into three mega rice varieties viz. Jyothi, Uma and Jaya is underway.

# Mandate

- 1. To evolve high yielding rice varieties having multiple tolerance to abiotic stresses like salinity, acidity and submergence suited to the coastal saline agro ecosystem.
- 2. To standardize appropriate management practices for yield enhancement of Pokkali rice.
- 3. To develop suitable integrated farming system model suited to the Pokkali tract so as to increase the income per unit area.

# About the wonder rice genotype Pokkali

Rice is the most important food crop of Kerala. The production of rice should increase by at least 40% in the next 25 years to keep pace with the growing world population. About 20% of the world's agricultural land and nearly 50% of all irrigated land is adversely affected by soil salinity. Moreover, soil salinization due to irrigation is becoming increasingly detrimental to agriculture.

In Asia alone, 21.5 million ha of land area is thought to be salt affected with India having 8.6 million ha. Rice is a salt sensitive crop but can grow in flooded condition. The only answer to extend rice cultivation for the ever growing population is to extend rice cultivation in the unfavorable water stagnated saline areas. For this purpose the saline tolerant gene complex (*Saltol QTL*) has to be introgressed into our high yielding rice genotypes. The *Saltol QTL* has been identified from Pokkali and is in wide utilization throughout the world.

Pokkali cultivation is the only natural organic farming system prevailing in the state, where no chemical fertilizers or plant protection chemicals being used. The special attributes of this wonder rice are,

- Pokkali is the most saline tolerant rice variety of the world and the donor of Saltol QTL for the international saline tolerant rice breeding programme.
- A wonder variety having "three in one" A variety blessed with tolerance to three abiotic stresses (salinity, submergence & acidity) in one variety.
- The only naturally producing organic rice of Kerala.
- Good medicinal value its bran is rich in oil and antioxidants like oryzanol, tocopherol & tocotrienols which is higher than that of popular medicinal njavara rice.
- Low glycaemic index and thus good for diabetic people
- Rich in Fe, K, Zn and Mn

This system of cultivation is unique in the world and is vanishing due to many practical problems and socio economic constraints. This precious genotype has to be conserved and this unique cultivation needs to be retained for keeping the sustainability of the ecosystem.

To conserve this special genotype and unique system of cultivation, the Rice Research Station, Vyttila had taken keen interest to get the Geographical Indication Registry Certification, and a logo for Pokkali rice and its value added products (2007), so as to market as a branded rice to fetch premium price for this pure organic product. Further, this Centre had also taken initiative to nominate the Registered Pokkali Farmer Group (Varappuzha-Kadamakkudy Jaiva Pokkali ICS) for the Plant Genome Saviour Community Award (2011-12) by the Protection of Plant Variety & Farmers' Right Authority, Govt. of India, with a cash award of Rs. 10.00 lakhs and a citation. This amount was intended to protect these valuable genotype through cultivation forever.

Pokkali Logo Geographical Indication Registry Certificate **Plant Genome Savior Community Award** Citation .

# **MAP OF POKKALI TRACT**



# The unique system of Pokkali Cultivation

# 1.Area and Distribution

The total area under saline soils of Kerala was estimated at 26,400 hectares with the following district - wise distribution.

SI.No	District	Area (ha)
1	Ernakulam	20,000
2	Alappuzha	3,000
3	Kannur	2,000
4	Other coastal districts	1,400
-	Total	26,400

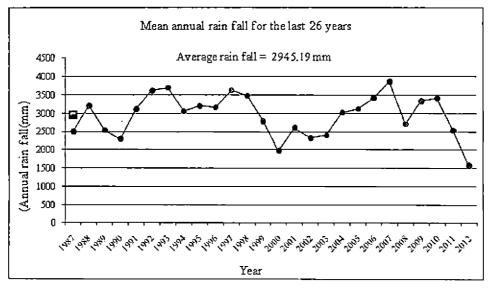
The Pokkali area under cultivation had steadily declined to the present level of about 8000 ha during the last two to three decades, mainly due to the large scale urbanization, conversion to non-agricultural purposes, and other socio economic constraints of pokkali cultivation.

# 2.Ecology:

As distinct from saline soils found elsewhere in India, the origin, genesis and development of these soils are under peculiar climatic and environmental conditions. These soils comprise low lying marshes and swamps situated near the mouths of streams and rivers not far from the sea. These soils are water logged and ill-drained and are subjected to tidal action throughout the year, with a tidal amplitude of about one metre. These lands in their natural state are overgrown with mangroves and other salt resistant plants.

# 3. Climate:

The average annual rainfall is more than 2900 mm, the major part of which is received in the months of June, July and August. The maximum day temperature varies from 27° to 34° and minimum temperature from 21° to 28° C. Humidity is often very high, recording more than 90%. Heavy rains occurring continuously for 10-15 days result in flash flooding, which is usual during June, July and August.



## 4. Soil and profile characteristics:

Soil is stiff impervious clay, rich in organic matter. It is bluish black in colour and is more than one meter deep. The soil is hard and creates deep fissures when dry and sticky when wet. The profile characteristics of soil at Vyttila are presented below.

# PROFILE CHARACTERISTICS OF 'POKKALI" SOIL

# a. PHYSICAL CHARACTERESTICS

Physical attributes	A Horizon	B Horizon	C Horizon
Depth	0-20 cm	20-50 cm	50-75 cm
Colour	Grey	Dark grey	Very dark grey
Structure	Blocky	Blocky	Puddled
Texture	Sandy clay	Clay loam	Clay loam
Consistency	Compact	Sticky	Very sticky
Permeability	Permeable	Less Permeable	Impermeable
	Depth Colour Structure Texture Consistency	Depth0-20 cmColourGreyStructureBlockyTextureSandy clayConsistencyCompact	Depth0-20 cm20-50 cmColourGreyDark greyStructureBlockyBlockyTextureSandy clayClay loamConsistencyCompactSticky

## b. MECHANICAL CHARACTERSTICS

SI.No.	Mechanical attributes	A	В	С
1	Coarse sand %	32.3	24.6	22.2
2	Fine sand	35.1	30.3	24.3
3	Silt	13.2	14.2	15,7
4	Clay	16.0	23.2	28.5

# c. CHEMICAL CHARACTERESTICS

SI.No.	Chemical attributes	A	В	С
1	Loss on ignition %	9.0	10.6	15.0
2.	Reaction (pH)	5.8	5.7	5.5
3.	Organic carbon %	1.3	1.9	2.1
4.	Total P <sub>2</sub> O <sub>5</sub>	0.009	0.006	0.053
5.	Total K,O	0.384	0.301	0.342
6.	Total F <sub>2</sub> O <sub>3</sub>	4.2	6.4	3.1
7.	Total Al <sub>2</sub> O <sub>3</sub>	14.9	9.1	12.1
8.	Total CaO	0.007	0.004	0.004
9.	Total Mg, O,	0.363	0.370	0.393
10.	E.C.(dS m <sup>-1</sup> )	1.5	2.2	2.2
	(J. IND. SOC. SOIL SCI - 18 (1) - 1970)			

With regard to nutrient status, the soil is very low in phosphorus, medium in nitrogen and high in potash. During summer months, due to ingression of salt water from the sea, the soil becomes saline. But when the salinity is washed off in heavy monsoon rains the inherent acidity of the soil regenerate. The soil is normally acidic, the pH being 3.0 to 5.5. The range of average pH and Electrical Conductivity of soil samples from the top of mounds and at 30 cm depth from the top at Rice Research Station, Vyttila after the receipt of pre monsoon showers is furnished below.

Soil sample	pH (1:2.5) range	EC (dS m <sup>-1</sup> ) range
From top of mound	3.40 - 4.35	12.4 - 16.5
From bottom of mound	5.20 - 6.55	15.9 - 24.2

Water soluble salts like sulphates and chlorides of sodium and manganese are present in high proportion. In dry conditions white encrustations of aluminium hydroxide also develop on soil surfaces. The electrical conductivity of the soil during summer months (Jan - May) varies from 12-24 dS m<sup>-1</sup> and the average salt content reaches up to 18 ppt (1.8%). During rainy season (June-August) water becomes almost fresh, salt content reduces to traces and electrical conductivity ranges from 4 to 6 dS m<sup>-1</sup>

# 5. Season and Cropping pattern

More than 95% of the total area is single cropped lands. In about 5% of the area, two crops are raised mainly by varietal mixture (Cherthala and N. Parur areas). More than 90% of single cropped 'pokkali' lands are cultivated during first crop season (Virippu) from May-June to September - October. In the rest of the area the cultivation is during second crop season (from August-September to December-January). The cultivation of rice crop in the second crop season with 'Orumundakan' is prevalent in stray patches at Muthukulam in Alapuzha district, Mathilakom in Thrissur district and Vadakkumpadu in Kannur district.

# 6. Method of cultivation:

Rice cultivation starts in the month of April by strengthening of outer bunds and setting up of sluices to control the level of water. The fields are then drained during low tide and the sluices are closed at high tide. When the soil becomes dry, it is heaped up to form mounds of about one meter base and half a meter height and the mounds are then allowed to dry and weather. With the onset of monsoon during May-June, the salt is washed off from the soil and the water with the dissolved salts is drained off from the field. Tops of mounds become free from salts very soon and sprouted seeds are sown.

# 7. Basic requirements of rice varieties for the region

Any substantial increase in rice production can be achieved only by evolving high yielding varieties suitable for peculiar soil conditions. Apart from high yield, the varieties should have the following basic requirements also.

- 1. Plant height not less than 125 cm.
- 2. Total duration not more than 125 days
- 3. Tolerance to submergence (Early seedling vigour)
- 4. Tolerance to salinity
- 5. Tolerance to acidity.

But now the plant height of varieties is limited to 125 -130 cm (semi tall stature) so as to prevent yield loss due to lodging and to facilitate mechanical harvesting which will compensate the labour shortage.

## a. Land races / Traditional varieties

'Pokkali' is the most popular variety used in this cultivation. Other varieties of the region are Choottu Pokkali, Chettivirippu, Cheruvirippu, Kuruka, Anakodan, Eravapandy, Bali, Orpandy etc.

# b. High Yielding Varieties

Eight saline tolerant high yielding varieties of rice have been released from Rice Research Station, Vyttila viz. VTL-1 to VTL-8. Out of these, VTL-1 to VTL-5 are tall varieties and cause considerable yield loss due to lodging. The last three varieties viz. VTL-6 to VTL-8 are semi tall and non lodging and the average yield is 3500 to 4000 kg/ha. Further, these varieties are amenable for mechanical harvesting also.

# 8. Seeds and sowing

Generally a high seed rate of 100 kg ha<sup>-1</sup> is being recommended in view of the possible loss due to flooding or poor germination and plant stand. Sprouted seeds are generally used for sowing. For sprouting of seeds, a special method was adopted. The seeds were tightly packed in country baskets made of

plaited coconut leaves, the inside of which lined with teak or karingotta leaves. These baskets were then immersed in freshwater for 15 to 18 hours after which they were taken out and stored in shade.

When the soil and weather conditions become favorable for sowing, the baskets containing seeds are resoaked for 3-6 hours before sowing. The mounds in the field are then raked and the top leveled. The sprouted seeds are sown on the top of the mounds which act as nursery *in situ*.

# 9. Seedling establishment and aftercare

The 'pokkali' varieties have early seedling vigor and they attain a height of 40 - 45 cm in 30 - 35 days. At about this stage, when field conditions become favorable the mounds were cut in to pieces with a few seedlings intact with a clod of earth, which were uniformly spread in the field. The seedlings being tall enough, survive in the flooded field condition. The clods of earth attached to the clumps give anchorage to the seedlings.

Generally, manuring or plant protection operations are not done by the farmers. Management of water level in the field is the only operation attended to after spreading seedlings. Standing water minimizes lodging and helps, to keep the tall plants erect and therefore water level is kept constant with the growth of plants.

In pokkali cultivation where no chemical fertilizers are added, tidal ingress anyhow, plays an important role in maintaining the nutrient status of the soil. In addition, a plethora of microorganisms flourish in the system which ultimately keep the soil very productive. But more significant is the annual transition from low saline to high saline phase such that the stevohauline fauna and flora particularly, the profuse floating vegetation dies out, decomposes and add on to the soil fertility. Together with decaying paddy stubbles retained in the field, the subsequent prawn filtration / farming is very much benefited by this form of organic recycling. Conversely the organic materials excreted by the fish and prawns and their remains are advantages for plant growth. Interestingly, even the simultaneous culture of fish / prawn along with rice is possible only because no plant protection measures are adopted in this area. Thus the integrated farming of rice with fish / prawn in the pokkali tract is an environment friendly, economically viable and sustainable enterprise.

# 10. Weeds of Pokkali region

Aquatic and semi-aquatic weeds occur in plenty during crop season. They get dried-up and decayed in the off-season during March to May. The important weeds are Echinochloa crusgalli, Eliocaris fistula, Fimbristyllis milliaceae, Monochoria vaginalis, Valisnaria spiralis, Nymphea sp., Marsilea quadrifolia, Astiracantha longifolia, Lymonophylla heretophylla, Sphenoclea zeylanica, Cyperus difformis, Ludwigia octovalois etc.

In addition, floating migratory weeds also create problems in cultivation. They are:

## 1. Salvinia åuriculata

## 2. Eichornia crassippes

But during high saline phase, the floating weeds and the enormous microflora and fauna decompose and adds to the soil fertility.

## 11. Pest and disease management

No major disease has been noticed in 'pokkali' crop. But there are three major pests which may occur in very serious form depending on weather conditions.

- 1. Stem borer Tryporyza incertulas
- 2. Leaf roller Cnaphalocrosis medinalis
- 3. Rice bug Leptocorisa acuta

Generally, the pest and disease attack in pokkali fields is below the threshold level. The prevalence of natural enemies and predators, varietal resistance, single cropped system etc. can be attributed to the low incidence of pests and diseases. The farmers are reluctant to use pesticides with a view to cause toxicity to the fishes and prawns.

There are some other non-insect pests, which are particular to pokkali crop. They are fishes, tortoises, rats and birds (sparrows, doves and parrots). Fishes of the genera *Ambassis, Anabas, Channa* etc. feed on the sprouting seeds (radicle and plumule and damage the seedlings). By burrowing the mounds and sprawling over them, tortoise also seriously damages the seedlings. The damage by rats (*Ratus norveageans*) starts from early seedling stage to harvest. Doves and sparrows cause damage at the time of sowing and after flowering of the crop. While the sparrows feed on the single grains on the panicle, the parrots carry away

# **Pokkali Cultivation**



Strengthening outer bunds



Mound Formation



Special seed packing



Germinated seeds sown on raked mound surface



Drying the field



Salts are washed off by rain water

- Seeds packed in special baskets- plaited with coconut leaves-lined with banana / teak leaves
- Soaked for overnight
- Sprouted seeds remain ' Quiescent' for > 30 days





Pasting the seeds with mud paste



Seedlings ready for dismantling



**Dismantled seedlings** 



Water Regulation in Pokkali fields



Seedlings on mound surface



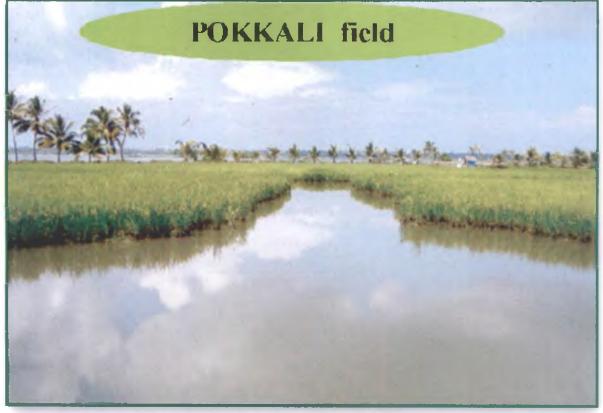
**Dismantling of seedlings** 



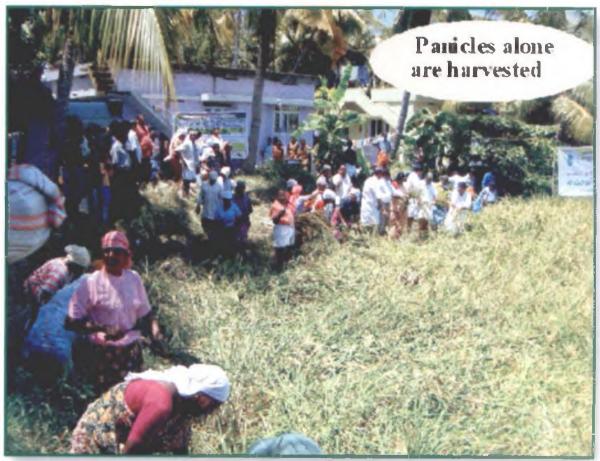
Established seedlings after dismantling



Pokkali field at active tillering stage



Pokkali field nearing maturity



Harvesting of Pokkali



Prawn catch after rice



Tiger Prawn

the panicles as such. Removal of nests from the neighboring areas is the present method of their control.

Recently, Purple moor hen (*Porphyrio porphyrio*), a migratory bird in large numbers causes extensive damage to rice crop by nesting inside the field, which can only be scared away to a limited extent by the use of reflective tapes and installing bird scarer units.

# 12. Harvest

The crop matures in about 110 - 120 days. Since, the time of harvest coincides with the north-east monsoon and prevalence of waterlogging, the ear heads alone are harvested, leaving major portion of the straw in the field. Average yield of rice is only 1500 - 2000 kg per hectare, when traditional varieties are grown. Average cost of cultivation at present rate works out at Rs. 30,000/- per hectare. Margin of profit is very narrow and it often involves the risk of total crop damage also. But the cultivation of semi tall non lodging high yielding varieties with sufficient plant population per unit area can yield about 3500 - 4000 kg/ha without the addition of chemical fertilizers or plant protection chemicals. Though the cost of production has increased drastically due to high wage rate, the high yield of these varieties compensated the cost of production and the farmers get a reasonable profit from the rice cultivation.

# 13. Mechanization of the region

There is no mechanization for the pokkali farming except for threshing. Water logging and peculiar soil physical properties restrict the use of usual heavy agricultural implements in land preparation or combined harvesters. The ridges of 30 cm taken at 40 cm apart were found equally good with the traditional mound preparation. This will explore the feasibility of mechanization in future. However, initial evaluation of floating harvester specially designed for pokkali region has been found to be encouraging.

# 14. Science in "Pokkali" cultivation

# (1) Advantages of forming mounds

- (a) Where water is available in plenty, washing is the easiest and cheapest method for reclaiming saline soil. Kerala receives more than 3000 mm annual rainfall, the major part of which received from June to August. This heavy rainfall is utilized for washing off salts from soil. The process of washing is expedited by throwing the field into mounds, which also increases the surface area, thereby making washing more easy and perfect.
- (b) Due to impeded drainage under coastal condition, fields become flooded as soon as heavy rains set in. The mounds with about half a meter height, serve as nursery 'in situ' and thus save the young seedlings from total submergence.
- (c) Once the monsoon commences, the fields will remain flooded with 45-50 cm of standing water. Under such a condition, normal system of transplanting is impossible. When mounds are leveled the clumps of seedlings get fixed due to weight of soil attached to the clumps.

# (2) Sprouting of seeds in plaited coconut baskets

The sprouted seeds remain viable and *quiescent* for 30 - 40 days, so that it can be used at any time when the field conditions become favorable. It is achieved due to the high pressure and anaerobic condition developed within the tightly packed baskets.

When seeds in tightly packed baskets are soaked for 12-15 hours, expansion of seeds by imbibition of water creates pressure and temperature inside the basket. When the excess water in the basket is drained off, atmospheric air (oxygen) enters in the interspaces. The seeds start sprouting utilizing available moisture and oxygen inside the basket. Sprouting of seeds and respiration of seeds and sprouts (radicle) exhaust the available oxygen inside the basket. Carbon-di-oxide exhaled by the sprouts creates an anaerobic condition inside the basket and prevents further growth of the radicle. The sprouts (radicle) thus remain *quiescent.* 

On the periphery of the basket, the sprouts having direct contact with atmospheric oxygen continue to grow and form a matting on the baskets. This condition results in preventing the contact of the seeds inside the basket with atmospheric oxygen, thus contributing for their *quiescent* condition.

When re-soaking is done, the Carbon-di-oxide inside the basket is displaced by water. On draining the basket again atmospheric air (oxygen) gets in, which expedites further growth of sprouts.

#### Prawn filtration

Prawn filtration is a traditional technique of prawn culture developed by innovative farmers of the pokkali region. It is practiced in pokkali fields and other low lying backwater areas having a tidal amplitude of about one meter.

After the harvest of paddy, by the end of October, the fields are allowed to have free exchange of water. Floating weeds like Salvinia and Eichornia are removed from the fields, the outer bunds strengthened, and the sluice gates are fixed in places where there is medium flow of water from the canal or backwater to the fields. After these preliminary preparations, water is let into the fields during each high tide at night, through the sluice, where a hurricane lamp is hung for luring in prawns. During low tide water is let out through a bamboo screen which prevents escape of the fish and prawns already entered into the field and brings down the water level, so that water can again be taken in during high tide. The actual fishing operation starts by the middle of January coinciding with the lunar phase. The prawn filtration net is also peculiar. It is conical in shape having a total length of 4.5 m with a trap system in the middle and a valve at the cod end for easy collection of the catch. The net is fabricated in close mesh, tied to a wooden frame and fitted to the sluice with its cod and kept in the channel or backwaters outside. When the water is let out of the fields, during low tide the prawns and fishes carried along with water and are collected in the prawn filtration net. Luring in of prawns continues simultaneously along with fishing till the end of March, when the fields are finally drained for taking up paddy cultivation. The yield per ha in this practice for a period of five months is about 800-1000 kg. Prawns constitute about 80% of the catch. The species being Metapenaeus dobsoni (65%), Metapenaeus monoceros (10%), Penaeus indius (22%) and Penaeus monodon (3%) depending upon the season. Fishes comprise 20% of the harvest the main species being Tilapia sp., Mystus sp., Etroplus suratensis, Etroplus maculates, Mugil sp, Lates calcarifer, Anabas testudineus, Megalops cyprinoides, Elops saurus etc.

## Advantages of Prawn Filtration:

- 1. The investment is negligibly low. It is a source of subsidiary income to the farmers without much investment.
- 2. If the season is favourable, a good crop of rice ensures a good catch of prawns because residual plant material is also high which offers a rich feeding ground for the prawns.

# The present scenario of pokkali area and cultivation:

At present, the area under pokkali tract has reduced drastically into only 8000 ha due to urbanization, conversion to non agricultural uses, low price of paddy, high wage rate and non availability of labourers for cultivation practices etc. The substantial income from the subsidiary prawn cultivation tempted the farmers to concentrate on prawn cultivation alone / round the year prawn cultivation without paddy cultivation. This practice caused several diseases to prawn and thereby the income has reduced drastically. Thus the farmers could realize that rice cultivation is an integral part of pokkali farming and the farming system involving rice cum fish followed by prawn is the sustainable farming system in the pokkali tract.

# **RESEARCH ACCOMPLISHMENTS**

#### A. Rice Breeding

The pokkali ecosystem has complex abiotic stress problems - salinity, acidity and submergence. Any variety developed for this ecosystem should be tolerant to these abiotic stresses. The varietal requirements for the pokkali ecosystem are

- Plant height not less than 125 cm
- Plant duration not above 125 days
- Tolerance to salinity, acidity and submergence
- Should possess dormancy from 2 weeks to one month

# a. Introduction of saline tolerant accessions

# Germplasm collection and utilization

As a first step of rice varietal improvement, land races from different pokkali ecosystem like Chellanam, Kumbalangi, Vypin, Kadamakkudy, Parur, Cherai, Thuravoor, Pattanakkad, Cherthalai etc were collected and evaluated the performance in the Pokkali tract. The land races like Pokkali, Cheruvirippu, Chettivirippu, Kuruka, Karutha kuruka, Ponkuruka, Anakomban, Mundakan etc are some among them. Apart from the local collections, saline tolerant accessions from other states and countries were also introduced, evaluated and utilized for the breeding programme. Further, they are being maintained in the germplasm bank. The national accessions

include CSR-10, SR-26-B etc. The international accessions are IR-5, Nona Bokra etc. A saline tolerant germplasm of about fifty five accessions are now being maintained in the germplasm bank.

# **b.** Pureline Selection

Selection in the local varieties - both 'mass' and 'pureline' selection was attempted from the very beginning of the station. Pureline selection was started in the two widely cultivated local varieties viz. Choottu pokkali and Cheruvirippu.

The first high yielding saline tolerant rice variety VTL-1 was evolved by pureline selection from Choottu pokkali and released for cultivation by the Department of Agriculture. This variety has 10 - 15 % higher yield than the local variety.

1	Сгор	Rice (Oryza sativa)
2	Variety	VTL-1
3	Method of breeding	Pureline selection
4	Source / parent / pedigree	Choottu pokkali
5	Year of release and authority	Kerala State Seed Committee, before 1974
6	Evolved at	RRS, Vyttila
7	Specific advantages / features	Photo insensitive tall lodging variety, medium duration (115 days),good cooking quality, tolerant to salinity, acidity and submergence. Donor of <i>Saltol</i> QTL
8	Key characters for identification	Tall lodging plants (160 - 170 cm) with kneeing ability. Grains are long bold and red with special taste.
9	Average yield	1500 kg/ha
10	Realizable yield	2000 - 2500 kg/ha
11	Area of adaptation	Pokkali tract & other coastal saline ecosystem
12	Tips on cultivation	Suitable for kharif season as organic rice
13	Scientists responsible for developing the variety	Department officers

The second high yielding saline tolerant rice variety VTL-2 was evolved by pureline selection from Cheruvirippu and released for cultivation by the Department of Agriculture. This variety has 10 - 15 % higher yield than the local parent variety.

Cran	
	Rice (Oryza sativa)
Variety	VTL-2
Method of breeding	Pureline selection
Source / parent / pedigree	Cheruvirippu
Year of release and authority	Kerala State Seed Committee, before 1974
Evolved at	RRS, Vyttila '
Registration / Notification details	Not available
Specific advantages / features	Photo insensitive tall lodging variety, medium duration (115days),good cooking quality, tolerant to salinity, acidity and submergence.
Key characters for identification	Tall lodging plants (170-180 cm) with kneeing ability. Tip awned grains are long bold and red with special taste.
Average yield	1750 kg/ha
Realizable yield	2500 - 3000 kg/ha
Area of adaptation	Pokkali tract & other coastal saline ecosystem
Tips on cultivation	Suitable for kharif season as organic rice
Scientists responsible for developing the variety	Officers of the Department of Agriculture
	Method of breeding Source / parent / pedigree Year of release and authority Evolved at Registration / Notification details Specific advantages / features Key characters for identification Average yield Realizable yield Area of adaptation Tips on cultivation Scientists responsible for

# c. Hybridization and selection

The rice hybridization programme commenced at the Rice Research Station, Vyttila, during 1975-76 period so as to combine the yield of released high yielding varieties and abiotic stress tolerances of the pokkali land races. The first high yielding variety evolved by combination breeding was VTL-3 by crossing VTL-1 and Taichung Native-1.

1	Crop	Rice (Oryza sativa)
2	Variety	VTL-3
3	Method of breeding	Hybridization and selection
4	Source / parent / pedigree	VTL-1 X TN 1
5	Year of release and authority	1987, by the Kerala State Seed Committee
6	Evolved at	RRS, Vyttila
7	Registration / Notification details	IC No.263761
8	Specific advantages / features	Photo insensitive tall lodging variety, medium duration (115days),good cooking quality, tolerant to salinity, acidity and submergence.
9	Key characters for identification	Tall lodging plants (170 - 180 cm) with kneeing ability. Tip awned grains are long bold and red with special taste.
10	Average yield	2000-2500 kg/ha
11	Realizable yield	3000 - 3500 kg/ha
12	Area of adaptation	Pokkali tract & other coastal saline ecosystem
13	Tips on cultivation	Suitable for kharif season as organic rice
14	Scientists responsible for developing the variety	Sri.George,T.U., Professor Sri.Tomy,P.J., Professor

Another rice hybridization programme commenced at this centre during 1980-86 period to combine the yield of high yielding rice varieties developed by IRRI, Philippines and the abiotic stress tolerances of the pokkali land races. The second high yielding variety evolved by combination breeding was VTL-4 by crossing the land race Chettivirippu with IR 4630-22-2-17.

1	Crop	Rice (Oryza sativa)
2	Variety	VTL- 4
3	Method of breeding	Combination breeding
4	Source / parent / pedigree	Chettivirippu X IR 4630-22-2-17.
5	Year of release and authority	1993, by the Kerala State Seed Committee
6	Evolved at	RRS, Vyttila
7	Registration / Notification details	IC No.263765
8	Specific advantages / features	Photo insensitive, medium duration(120-125 days) variety with good cooking quality. Tolerant to salinity and soil acidity, escapes flash flood and water stagnation. Tolerant tomajor pests and diseases.
9	Key characters for identification	Tall plants (160- 165 cm) having lodging habit and kneeing ability. Grains are straw coloured, medium bold with red kernel.
10	Average yield	3250 kg/ha
11	Realizable yield	3500 - 4000 kg/ha
12	Area of adaptation	Pokkali tract & other coastal saline ecosystem
13	Tips on cultivation	Suitable for kharif season as organic rice
14	Scientists responsible for developing the variety	Sri.George,T.U.,George,K.M, Shylaraj.K.S.,Tomy,P.J. & Reena Grittle Pinhero

# c. Mutation Breeding

Generally, the people of Kerala prefer red rice only. Considering the importance of export of pokkali rice being organic, a mutation breeding programme was initiated at this centre during 1984 - 86 period to develop a white kernelled rice variety suited to pokkali ecosystem and thereby could evolve the <sub>b</sub> mutant high yielding variety VTL-5 from the widely accepted variety Mahsuri.



VTL - 1





VTL - 2

VTL - 4



VTL - 5



VTL - 6



**VTL - 7** 



VTL - 8

1	Crop	Rice (Oryza sativa)
2	Variety	VTL-5
3	Method of breeding	Mutation breeding
4	Source / parent / pedigree	Mahsuri
5	Year of release and authority	1996; by the Kerala State Seed Committee
6	Evolved at	RRS, Vyttila
7	Registration / Notification details	IC No.470140
8	Specific advantages / features	Photo insensitive tall lodging variety, medium duration (115days), good cooking quality, tolerant to salinity, acidity and submergence.
9	Key characters for identification	Tall lodging plants (140-150 cm) with kneeing ability. Grains are having golden seed coat, medium bold with white kernels. The rice has a special taste.
10	Average yield	3250-3500 kg/ha
11	Realizable yield	3500 - 4000 kg/ha
12	Area of adaptation	Pokkali tract & other coastal saline ecosystem
13	Tips on cultivation	Suitable for kharif season as organic rice
14	Scientists responsible for developing the variety	Sri.George,T.U.,George,K.M, Dr.Shylaraj.K.S., and Dr. Sasidharan, N.K.

# e. Development of semi tall non-lodging high yielding varities

When the labour availability became a problem and labour wages enhanced for agricultural workers, there was a demand from the farmers to evolve a semi tall high yielding rice variety having tolerance to the multiple abiotic stresses of the ecosystem - salinity, acidity and submergence so as to facilitate mechanical harvesting. Hence a breeding programme was started during 1992-93 period to develop a semi tall non lodging high yielding variety amenable for mechanical harvesting. Thus VTL - 6 was developed from the cross Cheruvirippu/ IR 5//Jaya.

1	Сгор	Rice (Oryza sativa)
2	Variety	VTL-6
3	Method of breeding	Hybridization and selection
4	Source / parent / pedigree	Cheruvirippu / IR 5 //Jaya
5	Year of release and authority	2004, by the Kerala State Seed Committee
6.	Evolved at	RRS, Vyttila
7	Registration / Notification details	IC No.470141
8	Specific advantages / features	Medium duration (105-110 days), good cooking quality, non photosensitive, tolerant to salinity, acidity and submergence. Good hulling % (80)
9	Key characters for identification	Generally semi tall (115-120 cm) non lodging plants with light green leaves. Auricles absent. Grains with golden seed coat and red kernels.
10	Average yield	3500 - 4000 kg/ha
_ 11	Realizable yield	5000 - 6000 kg/ha
12	Area of adaptation	Pokkali tract & other coastal saline ecosystem
13	Tips on cultivation	Suitable for kharif season as organic rice
14	Scientists responsible for developing the variety	Dr. Shylaraj K.S., Prof. George K.M, Dr. Sivan Pillai, Dr. N.K. Sasidharan, Dr. Sreekumaran V. and Dr. K.Anilakumar

With the objective of developing a semi tall non lodging high yielding white kernelled rice variety suitable for export purpose, the variety, VTL-7 was developed. The parentage is IR-8 x Patnai 23.

1	Сгор	Rice
2	Variety	VTL-7
3	Method of breeding	Hybridization and selection
4	Source / parent / pedigree	IR-8 x Patnai 23
5	Year of release and authority	2006, by the 23rd Kerala State Seed Committee
6	Evolved at	RRS, Vyttila
7	Registration / Notification details	IC No.583747
8	Specific advantages / features	Medium duration (115- 120 days) variety with long slender white kernelled grains. It is a non photosensitive variety tolerant to salinity, acidity and submergence.
9	Key characters for identification	Generally semi tall (115-120 cm) non lodging plants with erect green leaves. Long slender grains with golden seed coat and white kernels.
10	Average yield	4000 - 4500 kg/ha
11	Realizable yield	5000 - 6000 kg/ha
12	Area of adaptation	Pokkali tract & other coastal saline ecosystem
13	Tips on cultivation	Suitable for kharif season as organic rice
14	Scientists responsible for developing the variety	Dr.Shylaraj.K.S., Prof.George,K.M, Dr.Sivan Pillai, Dr. N.K.Sasidharan, Dr.Sreekumaran.V and Dr.K.Anilakumar

For the slightly deep waterlogged areas, there was a demand for a slightly tall non lodging high yielding variety. Hence the variety VTL -8 was evolved from the cross IR 47310-94-4-1 X CSR-10.

1	Crop	Rice
2	Variety	VTL-8
3	Method of breeding	Hybridization and selection
4	Source / parent / pedigree	IR 47310-94-4-1 X CSR 10
5	Year of release and authority	2010, by the 25th Kerala State Seed Committee
6	Evolved at	RRS, Vyttila
7	Registration / Notification details	IC No.583748
9	Key characters for identification	Generally semi tall (130-135 cm) non lodging plants with drooping green leaves. The grains are medium bold, awnless with red kernel
10	Average yield	4100 - 4500 kg/ha
11	Realizable yield	5000 - 6000 kg/ha
12	Area of adaptation	Pokkali tract & other coastal saline ecosystem
13	Tips on cultivation	Suitable for kharif season as organic rice
14	Scientists responsible for developing the variety	Dr. Shylaraj.K.S., Prof. George K.M, Dr. Sivan Pillai, Dr. N.K. Sasidharan, Dr. Sreekumaran.V and Dr.Alice Antony

# The nutraceutical properties of Pokkali rice

The medicinal properties of pokkali rice is always praised by pokkali farmers. But the actual component contributing the medicinal values were not studied. Hence a scientific investigation was undertaken at the Rice Research Station, Vyttila to reveal the nutraceutical properties of pokkali rice. The pokkali bran was found to be nutritionally rich in bran oil and antioxidants like oryzanol, tocopherol, tocotrienols etc. compared to the most popular varieties like Jyothi and Uma. Oryzanol is famous for its antioxidant, anticancer and antilipidaemic property. Tocopherols and tocotrienols are vitamin E analogues which have antioxidant and anticancer properties. These contents were also compared with the medicinal rice njavara. It was observed that these contents are high in pokkali rice bran. Further, the pokkali rice is rich in micronutrients like Fe, Zn, Mn and B. Hence mild polishing is only recommended for Pokkali rice so as to utilize the medicinal properties of rice bran. This study could also reveal the high medicial values of the two scented rice varieties of kerala viz. Jeerakasala and Gandakasala. These varieties have high bran oil content, oryzanol, tocopherol and tocotrienol contents.

SI.No	Varieties	Oryzanoi mg/100g bran	Tocopherol mg/100g bran	Tocotrienol mg/100g bran
1	Jyothi	112.1	0.44	1.22
_ 2	Uma	138.7	0.48	2.23
3	Pokkali	202.9	2.26	5.13
4	Black njavara	149.4	1.85	4.44
5	Golden njavara	160.0	2.01	3.90
6	Gadakasala	178.7	6.17	5.15
7	Jeerakasala	219.0	5.20	8.18

# Antioxidant contents of pokkali rice bran

Bran oil and micronutrient content of pokkali rice

SI.No	Varieties	Fat content (%)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	B (mg/kg)
1	Jyothi	6.96	16.8	13.0	0.3	7.2	25.3
2	Uma	6.93	11.2	12.2	0.8	13.0	35.0
3	Pokkali	9.83	25.0	22.6	1.4	20.4	76.7
4	Black njavara	7.88	14.2	12.6	1.4	13.2	38.3
5	Golden njavara	7.43	28.8	14.9	8.6	19.4	56.7
6	Gandakasala	9.45	10.4	13.8	1.4	14.2	41.7
7	Jeerakasala	10.89	16.0	13.0	3.8	22.4	76.7

# B. Crop Management

Several experiments were conducted at the Rice Research Station, Vyttila to study the crop response to various chemical fertilizers and to standardize an optimum fertilizer dose to pokkali rice cultivation.

# Nitrogen Management

Extensive experiments were conducted using various form of nitrogenous fertilizers (urea, Ammonium sulphate, Ammonium nitrate, Calcium ammonium nitrate etc.) and different mode of application ( soil application, foliar spraying and deep placement in the soil etc.) from the very beginning and it was observed that there is variation with respect to the type of fertilizer and the mode of application.

Ammonium sulphate was observed as a better source of N than urea in pokkali soils. Better performance of ammonium sulphate may be due to the retention of NH4 ions by clay particles. Urea being highly water soluble is more liable in different types of losses. Further, deep placement of ammonium sulphate pellets at 5cm depth in the field after transplanting was found better than broadcasting the same dose as ammonium ions can be retained in the clayey soil of the rice root zone for a longer period.

# Phosphorus Management

Comparative efficiency of basic slag and super phosphate was assessed in a field experiment for three years from 1967 to 1970. Fifty and hundred kg of  $P_2O_5$  as superphosphate were compared with a 'no P 'and a'no fertilizer', control. Except for no fertilizer control, 30 kg each of nitrogen and potassium were given in the form of ammonium sulphate and muriate of potash respectively. Grain yield was significantly increased by the application of phosphate fertilizers. Basic slag and superphosphate were equally effective in the acid saline soils of Vyttila. Fifty kg  $P_2O_5$  was found better than 100 kg/ha.

Basic slag and super phosphate were found equally efficient as P sources. Grain yield considerably increased by P fertilizer application up to 50 kg  $P_2O_5$  ha-1, since the soil is highly deficient in P.

# Management of major nutrients

Different experiments were conducted to standardize an optimum dose of major nutrients for the pokkali cultivation and found that the application of 20kg N and 40kg  $P_2O_5$  ha-1 at the time of dismantling and distribution of seedlings was the best treatment which recorded 35% higher yield. Among the major nutrients, N was found to be the most efficient one in increasing grain yield followed by phosphorus and potassium in the decreasing order.

Application of 20 kg N and 40kg P2Os harl consistently increased grain yield under pokkali condition. The time of dismantling of mounds and distribution of seedlings in the field was found to be the best time of application of N and P fertilizers.

Foliar application of 17 kg ha-1 as ammonium phosphate (2% solution) was found effective for rice. For soil application double the dose (34 kg/ha) was required.

# Mounds v/s Ridges

As a part of the standardization of agro techniques, various land preparation methods, viz. mounds, ridges, shallow bed etc. were compared for plant stand establishment and yield of rice and found that mounds were superior in terms of yield of rice under pokkali conditions. However, ridges of size 30 cm taken at 40 cm apart was found to be equally good, which may be useful in developing some devise for the mechanization of land preparation in pokkali.

# Organic manures and bio-fertilizer

Efficiency of green leaf for increasing grain yield was tested in an observational trial. Fifteen percent increase in grain yield was obtained by the application of green leaves at 5t ha-1.

The efficiency of Blue Green Algae was tested in pokkali fields as a source of bio-fertilizer and concluded that it is not suitable for the agro-ecological situation of pokkali lands though blue green algae multiply rapidly.

# Soil amendments

Ameliorating effects of different levels of lime, saw dust and gypsom in the acid - saline soils were tested. The application of 1000 kg/ha lime along with 20kg N and 40kg P2O5 hat recorded the highest yield. But doses higher than 1000 kg/ha reduced the grain yield. Half the quantity of lime was applied on the mounds while sowing and other half at the time of dismantling and distribution of seedlings. Average yield increase was 21% more than "no lime" control.

Saw dust @ 300kg ha-1 as an amendment was not beneficial in Pokkali soils. Application of 500kg gypsum ha-<sup>1</sup> in ten equal splits did not increase grain yield of rice in Pokkali soils.

Though the soil is having a very low pH, ranging from 3.5 to 3.8, more than 1000kg lime hat is not found to increase grain yield. Application of 1000kg lime ha-1 increases grain yield by 21%. Detailed investigations are required to assess the cause for reduction in yield with higher doses of lime. As grain yield obtained by liming is not economical, varietal screening of the existing high yielding rice varieties for their adaptability to the agroecosystem is warranted.

# Nutrient flux of Pokkali soil

Forty seven padasekharams in the Pokkali tract were monitored and the nutrient flux assessed

Parameters	Low saline phase Rice Harvesting Stage	High Saline phase Prawn Harvesting Stage
	2.62 to 5.97	3.31 to 6.46
ECdSm <sup>-1</sup>	0.01 to 7.80	0.10 to 9.80
00%	0.45 to 2.90	0.48 to 2.79
P kg ha <sup>-1</sup>	0.24 to 88.24	12.32 to 119.24
K kg ha 1	13 to 1777	52 to 1086
Ca mg kg <sup>-1</sup>	76 to 256	60 to 218
Mg mg kg <sup>-1</sup>	9 to 20	6 to 8
S mg kg <sup>-1</sup>	8 to 6846	3 to 3625
Zn mg kg <sup>-1</sup>	2 to 173	2 to 125
Cu mg kg <sup>-t</sup>	2 to 13	1 to 10
Fe mg kg <sup>-1</sup>	171 to 232	172 to 2028
Mn mg kg 1	2 to 26	1 to 34
B mg kg <sup>-1</sup>	0.13 to 0.75	Trace to 1.20
Al mg kg <sup>-1</sup>	0 to 866	4 to 289

The soil pH of the padasekharams in the pokkali tract ranges from 2.62 to 5.97 indicating that the soils are ultra acidic to moderately acidic during the low saline phase. While during the high saline phase the pH ranges from 3.31 to 6.46 indicating ultra acidic to slightly acidic condition.

The salinity as indicated by the electrical conductivity during both the phases range from very low to high. The EC goes up to 7.8 dS m<sup>-1</sup> during the low saline phase and up to 19.5 dS m-1 during the high saline phase thus indicating the salinity prone condition in the pokkali tract.

This is a highly organic rich zone, the values being 0.45 to 2.90% in the low saline and 0.48 to 2.79% during the high saline phase. There is not much variation in the organic carbon build up in the soil, between the two phases.

Analysis results indicate that under highly acidic conditions of less than pH 5 there is high Al toxicity condition resulting in Mg deficiency in all the soil samples.

The organic carbon build up in the soil is steady both under the low and high saline phase. The P and K status ranges from low to very high throughout. The S, Zn, Cu, Fe and Mn is also high in these soils.

Dynamics of hazardous chemicals/heavy metals

Water and soil samples were collected from 6 locations during the high saline phase at the time of prawn filtration and the dynamics of hazardous chemicals /heavy metals were assessed.

Parameters	Kumbalam	Ezhupunna	Chellanam	Kadamakudy	Paravur	Njarakkal	Toleran	ce Limit
							Irrigation Water	Drinking Water
Ni (mg L <sup>-1</sup> )	0.33	2.46	0.51	0.14	0.39	0.25	0.20	
Pb (mg L <sup>-1</sup> )	0.17	0.12	0.32	0.12	0.18	0.51	5.00	0.05
As (mg L <sup>-1</sup> )	NIL	NIL	NIL	NIL	NIL	NIL	0.10	0.05
B (mg L <sup>-1</sup> )	NIL	NIL	NIL	NIL	NIL	NIL	0.75	-
Al (mg L-1)	NIL	NIL	NIL	NIL	NIL	NIL	5.00	-
Zn (mg L-1)	0.05	0.06	0.05	0.06	0.07	0.07	2.00	5.00
Cu (mg L <sup>.1</sup> )	0.04	0.05	0.04	0.04	0.05	0.05	0.20	1.00
Fe (mg L <sup>.,</sup> )	0.02	0.03	0.03	0.02	0.06	0.01	5.00	0.30
Mn (mg L <sup>.1</sup> )	0.04	0.04	0.04	0.04	0.05	0.05	0.20	0.10
Residues of OP, OCI, SyPy(pgL <sup>-1</sup> )	Not detected	0.50	-					

The results of analysis indicate that the water source from the 6 locations were not having hazardous chemicals /heavy metals except for Nickel. The content being less than the tolerance limit for irrigation in the case of Lead, Arsenic, Boron, Aluminium, Zinc, Copper, Iron and Manganese. Nickel was high in 5 locations ie. more than 0.2 mg L<sup>-1</sup> the tolerance limit for irrigation water.

The pesticide residues of organo phosphorous, organo chlorine and synthetic pyrethroid compounds were not detected in these water and soil samples. The detection limit being 0.5  $\mu$ g L<sup>-1</sup>.

Soil status of major, secondary and micro nutrients

Parameters	Kumbalam	Ezhupunna	Chellanam	Kadamakudy	Paravur	Njarakkal
pH	6.26	6.05	6.00	5.81	6.21	6.20
EC dSm <sup>-1</sup>	2.90	2.60	2.40	3.40	4.00	3.60
OC%	1.68	. 0.52	2.03	2.08	1.84	0.63
K(kg/ha)	652	265	1210	888	726	496
P(kg/ha)	64	79	62	134	86	95 ·
Ca(mg kg <sup>-1</sup> )	583	331	892	545	734	381
Mg(mg kg <sup>-t</sup> )	· 101	63	129	. 66	124	73
S(mg kg <sup>-1</sup> )	92	341	379	807	715	657

Zn(mg kg <sup>-1</sup> )	2.25	0.72	0.54	20.42	4.74	34.90
Cu(mg kg <sup>-1</sup> )	0.48	0.27	0.61	0.88	0.48	0.34
Fe(mg kg <sup>-1</sup> )	262	127	195	268	220	235
Mn(mg kg <sup>-1</sup> )·	4.84	0.38	1.64	0.96	0.93	2.88
B (mg kg <sup>-1</sup> )	2.06	2,96	2.67	2.23	2.58	2.55
Al (mg kg-1)	0.41	0.23	0.61	1.86	6.65	0.44

The soil samples were having pH more than 5.5 (range being 5.81 to 6.26) and thereby no solubility of Al and no toxic effect (range 0.23 to 6.65 mg kg<sup>-1</sup>). In addition there was availability of Mg ( range 63 to 129 mg kg<sup>-1</sup>).

The EC was in the saline phase (2.40 to 4.0 dS m<sup>-1</sup>) and the organic carbon status ranging from 0.52 to 2.08 %. The P, K, Ca, Mg & S status was high at all the sites of sample collection indicating that these pokkali fields are suitable for organic rice production.

With regard to the micronutrient status, the Zn was deficient at 2 locations, Ezhupunna and Chellanam and at a toxic level at 2 locations Kadamakudy and Njarackkal. Cu was deficient at all the locations. Fe content was high and B sufficient at all the locations. Mn was deficient at 3 locations Ezhupunna, Kadamakudy and Paravur.

## Permanent Manurial Trial:

The permanent manurial trials indicated that the application of inorganic fertilizers with or without lime do not have any positive influence on grain yield of pokkali varieties. The pokkali fields are highly suited for organic farming. There is percentage yield reduction due to the application of chemical fertilizers in the pokkali soil . Thus the fertilizer recommendation of 20:40:0 kg N: P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O ha<sup>-1</sup> for pokkali rice was withdrawn from the package of practices recommendations, Kerala Agricultural University, based on the response studies conducted at this centre. This has helped to declare the entire pokkali tract as fully "organic zone"

Sl. No.	Treatment	Mean grain yield kg/ha (34 years) 1979 - 2012	% reduction over control
1	Control	2819	0.0
2	N alone	2765	-1.9
3	P alone	2862	1.5
4	K alone	2747	2.6
5	NPK	2738	-2.9
6	NPK + lime	2796	-0.8
• 7	NP + lime	2733	-3.1
8	P+ lime	2855	2.0
9	N(UMB)PK	2762	- 2.0
10	N(NCU)PK	2729	3.2
-	CD (0.05)	NS	

# Effect of continuous application of fertilizers on grain yield of Pokkali rice

For a sustainable rice production in the pokkali tract, there should not be any hindrance for the natural tidal inflow of water. This enriches the soil, without the application of any chemical fertilizers enabling the production of the renowned organic pokkali rice and sustaining the pokkali eco-system

Native flora of Azospirillum and P solubilizers were present in the pokkali soil while native K solubilizers were absent.

# C. Integrated Farming - Rice-fish/prawn integration in Pokkali fields.

The *Pokkali* rice-fish/prawn integration has been dealt with by several workers (Purushan, 1987 and Rajendran *et al.*, 1993). Most of the workers have described the different aspects of the prawn filtration system, in which seed shrimps and fishes were allowed to enter into the post harvest *Pokkali* fields through

tidal water and then trapped for short term culture. Prospects of simultaneous culture of fish species viz., *Etroplus suratensis, Oreochromis mossambicus, Cyprinus carpio, Labeo rohita, Chanos chanos* and *Mugil cephalus* with the *Pokkali* rice genotypes have been reported by Thampi (1993) and Rajendran *et al.* (1993). However the simultaneous rice-fish culture in *Pokkali* fields did not get the popularity as that of the sequential rice-prawn culture.

Prawn filtration in Pokkali fields starts with the strengthening of outer bunds and installation of sluice gates. Removal of weeds and desilting of canals, wherever necessary, are carried out for increasing the water holding potential (Rajendran, *et al.*, 1993). Brackish water carrying the shrimp seed ingresses and egresses regularly at desired levels during high and low tides respectively. While regulating the entry and exit of tidal flow, a large mesh conical bag net or closely packed bamboo screen is suitably installed in the sluice gate. This process being repeated cautiously during each tide, enables the shrimp and fish seed to get concentrated in the field (Purushan, 1986). The species auto stocked includes commercially important shrimps such as *Metapenaeus dobsoni, M.monoceros, Penaeus indicus* and *P.monodon*. These shrimps take shelter in between the decaying paddy stalks, which release nutrients to the shrimps and they grow (Purushan, 2002). The synergistic effect of all these and the congenial conditions prevailing devolve to form a rich niche of forage organisms to the growing shrimps that attain a marketable size within 3 to 4 months period

The actual harvesting of prawn starts by the middle of January coinciding with the lunar phase. The prawn filtration net is conical in shape. It has a total length of 4.5 metres with a trap system in the middle and a valve at the code end. When the water is let out of the fields, during low tide the prawn and fishes carried along with water and are collected in the prawn filtration net. Luring in of prawns continues simultaneously along with fishing till the end of March, when the fields are finally drained for taking up rice cultivation. The yield is about 800 to1000 kg ha<sup>-1</sup>. Prawns constitute about 80 per cent of the catch. The spp. being *Metapenaeus dobsoni* (65 %), *M. monoceros* (10%), *Penaeus indicus* (22%) and *P. monodon*(3%) depending up on the season. Fishes comprises 20 percent of the harvest. The main spp. are *Oreochromis mossambicus*, *Etroplus suratensis*, *Mystus sp.,Lates calcarifer*, *Anabas testudineus*, *Megalops cyprinoides*, *Mugil sp.* etc.

Prawn filtration is the traditional system of integrating rice and prawn/fish in Pokkali lands. The advantage of prawn filtration is that both the components of the system are mutually benefited. if the season is favourable a good crop of rice is ensured by a good catch of prawn on account of the high residual plant materials which offers a rich feeding ground for the prawns.

# Selective culture of Prawn

Prawn culture is the natural choice of cropping activity in Pokkali fields during the high saline phase considering the water quality parameters as given in the following table.

Quality parameters	Range	Mean
Field water pH	7 - 9	7.10
Dissolved oxygen (mg I <sup>-1</sup> )	5 - 8	5.30
Dissolved ammonia (ppm)	0.1 - 0.61	0.29
Total alkalinity (mg I-1)	32.0 - 48.0	38.60
Salinity (ppt)	4.1 - 12.2	7.90
Electrical conductivity (dS m <sup>-1</sup> )	8.6 - 15.8	11.70

# Water quality parameters of the selective culture of Tiger prawn in Pokkali fields

Selective culture of tiger/white prawn is an improvement over the traditional practice of prawn filtration. The success of traditional prawn filtration mainly depends on the availability of prawn seed. Often the percentage of quality prawn is very low affecting the income from the prawn filtration. Selective stocking of the paddy fields with fast growing varieties of prawn seed after preparing the field is an alternative. In selective stocking there is full control over the culture system. The native fish population in the fields can be completely eradicated. Stocking with desirable prawn seed and preplanned harvesting depending upon the growth of the prawn are possible.

A yield of 425 kg ha<sup>-t</sup> of prawn under selective culture, when fed with the live feed generated in the Pokkali fields is reported (Sasidharan, 2004). Average weight gain of 15.9 g in 50 days and 20 g at harvest in 74 days after stocking could be observed as given in the following table.

Growth parameters	Range	Mean
Length after 50 days (cm)	10.2 - 15.0	10.9
Weight after 50 days (g)	10.0 - 22.0	15.9
Length at harvest (cm)	15.8 - 19.3	18.2
Weight at harvest (g)	17.0 - 35.0	20.0

Growth parameters of Tiger prawn from selective culture in Pokkali fields

However, farmers opt for artificial feeds, which enhances the prawn yield and the production cost. The risk of crop failure is also high when the intensive farming techniques like higher stocking density and feeding with commercial feed formulations are resorted to.

# Rice- cum- fish culture

Integrated fish culture along with rice is feasible in Pokkali fields. Most of the Pokkali fields have peripheral channels, which are invariably required for the rice- fish dual culture. The depth of the channel may vary from 50 - 75 cm and the width from 100-200cm. The channels provide a safe habitat for fishes particularly during the low tide. A sluice with shutters facilitate exchange of water and regulation of water level, a removable net screen is provided in the sluice to prevent the escape of cultured fishes and the entry of undesirable organisms. The eradication of weed and undesirable fish can be achieved by applying bio - pesticides like mahua oil cake. Rice varieties and fish species with in the allowable tolerance limit for salinity alone can be selected for culture in Pokkali fields. Often the rearing of fish is continued even after the rice harvest, when the salinity may rise to higher values, in which case it is necessary to select species, which are euryhaline. In Pokkali fields, carps do not survive probably due to increased salinity and low oxygen concentration. Milk fish is susceptible to anoxic condition particularly during the post harvest decay of rice stubbles.

The utility of Oreochromis mossambicus as a suitable component of the rice-fish integrated farming system was amply illustrated by Rajendran et al. (1994). It's profuse occurrence, prolific breeding and shadowing effect on other species were dealt by Purushan (2002), while the possibilities of hormonal sex reversal and mono sex culture as a remedial measure have been reported by Roy (1998).

## Sustainable farming system.

The integrated farming system involving rice, fish and/or prawn followed in Pokkali fields is considered as a sustainable system. No way it interferes with the seasonal rhythm of climatic changes and the components of the system well mingle with nature. The tides regulate the salinity and pH of the soils and contribute to the fertility and productivity of the Pokkali soils. The components of the system compliment each other by organic recycling and generation of live feed.

The soil chemical characteristics of the Pokkali soil after the prawn harvest are given in the following table.

Soil chemical characters after	prawn harvest in Pokkali fields
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Soil chemical characters	Range	Mean
pH	5.70 <u>- 6.</u> 20	5.80
Electrical conductivity (dS m <sup>-1</sup> )	6.10 - 8.90	8.00
Organic carbon (%)	2.43 - 3.17	2.78
Available P (kg ha-1)	10.60 -15.10	11.90
Available K (kg ha-1)	314 - 1299	463
Available Na (kg ha <sup>-1</sup> )	3564 - 13440	548 <b>4</b>

The soil chemical characters after the prawn harvest denoted desirable changes in pH, organic carbon, available P and available K. However, the electrical conductivity and the available sodium registered considerable increase compared to the soil characters after the rice harvest.

The economic analysis of the integrated farming system point to it's economic superiority also. The production cost of rice under the Pokkali system of cultivation is comparatively lesser than the other rice ecosystems of the state. Inputs costs on fertilizer, pesticides and herbicides are nil in Pokkali fields. The expenditure on weed management, which usually costs 50 per cent of the production cost, is also lesser in Pokkali area.

Introduction of the fish component to the rice cultivation increases the production cost only marginally. The pokkali fields generally have deeper inner channels which require renovation before stocking. Removing weed fishes, cost of fingerlings and harvest operations are the other important items which require major expenditure. The direct effect of the fishes on reducing the cost on weeding and lowering of the cost on fertilizers were conspicuous by their absence in Pokkali fields. The tidal regulation of water depth, generally makes the Pokkali fields weed free. Similarly the Pokkali fields are basically fertile and fertility accretion rather than depletion is the general rule.

The economic analysis confirm that the integration of prawn and fishes with rice is the key for increasing the net returns and benefit : cost ratio of Pokkali fields. Hence, the simultaneous culture of high yielding Pokkali rice varieties and male tilapia during the low saline phase and subsequent prawn culture is a sustainable farming system which is socially acceptable, economically viable and ecologically sustainable.

#### Research on fisheries component

Taking into account the importance of fish-prawn culture, especially during the saline phase, a unit of fisheries research was established during the year 1976. Further, as part of the All India Co-ordinated Research Project on Brackish Water Fish Farming (ACRIP), seven grow out fish ponds of size ranging from 0.1 to 0.5 ha. were constructed. In addition, a few small nursery ponds were also constructed for fry rearing. The lab facilities were provided in a new building for Fisheries Unit constructed in this connection.

## Mandate of the fisheries research wing

Simultaneous culture of rice and fish during low saline phase, prawn culture during the high saline phase and fish/prawn culture in brackish water ponds were the lead functions of the Fisheries Unit.

## Integrated Farming System

Simultaneous rice fish culture in the saline tracts of pokkali fields poses several problems, characteristic to this region. The culture practices involve preparation of peripheral and diagonal trenches, eradication of unwanted fishes by applying suitable pesticides not harmful to rice, manuring and feeding, if necessary, to sustain growth. The fishes are stocked after paddy transplantation and the culture is prolonged even after paddy harvest so that fish attain marketable size. The major constraints in growing cultivable species like major carps and brackish water fishes like milkfish, grey mullet and pearlspot were the salinity rise, anoxic conditions due to decay of paddy stubbles, inhibition of light penetration and production of fish food organisms, and slow growth of fish. Hardy and resistant species like tilapia was found to be suitable in such conditions.

The prawn filtration is a traditional culture technique of efficient land utilization after paddy harvest. The fields are prepared by removing floating vegetation, strengthening bunds and fixing sluice gates to regulate water exchange. Water is let into the fields at high tide through sluice during night and at low tide, the fields are drained through bamboo screens to prevent escape of collected fish and prawns. The actual fishing operation commences by December and periodic harvesting is done on every 2 to 3 days before and after new moon and full moon till the middle of April. A conical bag net mounted on a rectangular frame, fixed in sluice, facilitates periodic removal of prawns collected at the cone end. The final harvesting is carried out after draining the fields. Eventhough, the farming system involves low investment and better returns, the catch is mainly

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composed of small sized prawns and the percentage of quality prawn fluctuates year to year. The production of prawn filtration ranged from 300 to 1600 kg/ha per season. The yield is unpredictable, mainly depend on natural recruitment. Information on the availability and seasonal variation of seeds of cultivable varieties of prawns and fish is hence essential.

Research to evolve ways to improve the traditional prawn filtration system has been approached in two directions. 1. Supplementary stocking of quality prawn, mainly white prawn *(Fenneropenaeus indicus)* in the prawn filtration fields with the aim of increasing the percentage contribution of high priced prawns. 2. Selective culture of prawns in pokkali fields. in the latter case, the fields are prepared by eliminating unwanted fish and weeds. Fields are manured and prawns are fed, if necessary. Prawn seeds are stocked and reared at varying densities and periods, depending upon the rate of growth. Multiple cropping is possible in regions where a favourable hydrographic condition, especially salinity, exists.

# Aquaculture in Fish Ponds

Investigation to evolve viable technology for the culture of brackishwater fish and prawn has been undertaken at this station. Experiments on monoculture and polyculture of brackishwater fishes, milkfish (Chanos chanos), grey mullet (Mugil cephalus), mullets (Liza sp) and Pearlspot (Etroplus suratensis) and tilapia (Oreochromis mossambicus) in various combination and management practices have been conducted. During the high saline phase, the ponds are utilized for culture of economically important prawns, white prawn (Fenneropenaeus indicus) and tiger prawn, (Penaeus monodon).

# Research highlights and accomplishments in fisheries

- A technology for prawn filtration during the saline phase as a follow up crop after rice has been standardised.
- Studies conducted on the effect of supplementary stocking of white prawn (Fenneropenaeus indicus) in traditional prawn filtration showed that the percentage of quality prawn can be increased by adopting this improved technique.
- Selective stocking of white prawn (F. indicus) at a stocking density of 50,000/ha in pokkali fields during the saline phase after paddy harvest gave an yield of 355 kg/ha/48 days without feeding. Adaptive trials in two farmers fields on the selective culture of white prawn @ 60,000/ha stocking density gave yields of 382 kg/ha/90 days and 552 kg/ha/83 days.
- In a pioneer work on simultaneous monosex culture of male tilapia (Oreochromis mossambicus) at a stocking density 3000 nos/ha and pokkali rice yielded 266 kg/ha/87 days fish with an average size of 201 g.
- Survey of seed resources of cultivable species of prawn and fishes revealed the availability of the post larvae of tiger prawn, *Penaeus monodon* in fairly good numbers during April to May in the Cochin backwater at Puthuvyppu, for the first time. It was also discovered that in the Cochin bar mouth area, post larvae of the economically important white prawn, *F. indicus* is available throughout the year with peak during March - September and that mullet seeds during April - November period. In the shallow mangrove at Puthuvyppu, seeds of milkfish were available during April - May and grey mullet seeds during June - August along with mullet seeds.
- In brackish water ponds, culture of white prawn, (F. indicus) @ 1 lakh per ha gave an yield of 704 kg/ha/62 days. In other culture trials yield of 320 kg/ha/79 days @50,000/ha without feed and 597 kg/ha/46 days and 649 kg/ha/52 days at stocking density of 1 lakh per ha were obtained.
- Culture of tiger prawn, P. monodon in brackish water ponds @ 20,000/ha with manuring and feeding gave a gross yield of 615.5 kg/ha/90 days.

- In monoculture of pearl spot (Etroplus suratensis) increase in stocking density from 25000 to 30,000/ha increased yields from 375 kg/ha/180 days to 1118 kg/ha/318 days. Experiments on supplementary feeding showed no significant difference.
- Polyculture of brackish water fishes mainly milkfish (C. chanos), grey mullet (M. cephalus), Pearlspot (E. suratensis) and mullet (Liza sp). at different combinations, densities and management practices showed that the yield ranged from 985 kg/ha/11 months to 2196 kg/ ha/13 months and in short term experiments 674 kg/ha/6 months to 1353 kg/ha/ 7 ½ months. Out of the different combinations, culture of milkfish (Chanos chanos) and grey mullet (Mugil cephalus) gave the highest production of 2136/kg/ha/yr and recorded maximum growth rate.
- Mixed culture of fresh and brackish water fishes in brackish water ponds during low saline phase yielded a production of 1446 kg/ha/4 months which is much higher than that obtained from culture of brackish water fishes alone in this area.
- Utilization of homestead ponds with sandy loam bottom in and around Cochin (100 200 sq m) by culturing grass carp, silver carp, common carp, catla, rohu, mrigal and milkfish gave yields ranging from 793 to 3932 kg/ha/yr.
- Detailed investigation of the physico-chemical and biological parameters of brackish water fish ponds were conducted. The zooplankton and macrobenthic fauna in the culture ponds were found to be composed of copepods, rotifers, cladocerans, crustacean nauplii, polychaetes, amphipods, gastropods, tanaedaceans and insect larvae. Zooplankton, especially copepods and crustacean nauplii dominated during the saline phase from January to May while benthic fauna did not show significant variation between high saline and low saline period.

# D. Research on cut flowers

The Rice Research Station, Vyttila is located at the centre of Kochi metro and there is huge demand for cut flowers like orchids, anthurium etc. Hence research work was initiated during 1996 period for the collection, screening, hybridization and mass propagation through mericioning of orchids. Orchid culture has been identified as a lucrative agri-business in India. However growing of orchids in India commercially is not organised and is still in the hands of hobbyist and few dealers who mainly depend on wild collections from forest to meet a large part of their foreign and local demands, due to which some of the orchids-growing areas are now without any orchid and very rare species are now facing the danger of depletion.

Dearth of quality planting material at reasonable price is another hurdle. At present, the planting material is imported from other countries at an exhorbitant cost. Moreover, the hybrids that they are prepared to spare will be the ones that have lost their competitive relevance. Planting materials of the latest hybrids reigning in the international auction markets will never be exported to buyer countries.

Moreover considering the new international patent policies on plants and plant products, the import of planting materials from other countries may very soon become an obsolete practice and so we must be ready with our own hybrids urgently.

In view of the economic importance and endangered status, orchids deserve special attention. Despite the fact that micro propagation protocols have been developed for several indian orchid species/varieties, development of meaningful orchid based cut-flower and pot plant industry in the country still awaits motivated entrepreneurs.

The major objectives of orchid research at RRS, Vyttila are the following

- To identify and collect species and varieties with already proven commercial qualities, free flowering habit and suitability to our climate as parents.
- To develop new hybrids of Dendrobium, Phalaenopsis and Vanda with high ornamental value

and commercial qualities for cut flower production and potted plant industry, thus reducing the need for import at an exhorbitant cost. Owing to considerable genetic diversity all these genera provide ample scope and has immense potential for intervarietal and interspecific hybridisation.

To standardize in vitro multiplication protocols of the selected hybrids which will help to overcome the problem of shortage of hybrid planting material in the consumer market.

# ACHIEVEMENTS

# a. Collection and screening of proven varieties of orchids

Fifty Dendrobium varieties / hybrids, twenty four Phalaenopsis hybrids and twenty Vanda hybrids were collected and screened for adaptability, longevity of inflorescence etc. and selected the adaptable varieties / hybrids for using as parents in hybridization and for mass multiplication through mericloning.

# b. Dendrobium hybridization

The selected dendrobium varieties were hybridized to evolve new hybrids suited to our ecosystem. A beautiful dendrobium hybrid could be evolved by crossing the *Kasim white* with *Burana sundae*. This hybrid will be multiplied and distributed for cultivation.

# c. Economically viable protocol standardization for commercial production

Research for the development of economic protocols for the meristem culture of Dendrobium, Phalaenopsis and Vanda are being standardized.

# E. MASS PRODUCTION OF TISSUE CULTURE BANANA AND ORCHIDS

Research on tissue culture production of banana and orchids was initiated along with rice research so as to do service to the farming community and to enhance the farm income. The station could evolve effective commercial protocols for the mass multiplication of virus indexed banana and orchid varieties / hybrids. The existing laboratory could be renovated and a new laboratory could be established with the funding from State Horticultural Mission, Kerala, Department of Agriculture and Co-operation, Govt. of India and ICAR Development Fund.

# a. Need for tissue culture in banana

Large number of banana varieties grown under diverse systems of cultivation is a unique feature of Kerala. Nendran, a signature variety for its excellent quality, is the only extensively grown commercial variety of banana in Kerala. Apart from this, varieties like Robusta, Kadali, Chenkadali, Palayankodan, Poovan and Njalipoovan are also cultivated as pure crop but on a limited scale. Being somewhat shade tolerant, these varieties are mainly spread on homesteads.

The propagation of banana is through suckers (vegetative means). A single plant gives a maximum of 5-10 suckers only. The low rate of multiplication limits this method severely. In the recent years, tissue culture propagation of banana through shoot tip as well as floral *aspices* has been utilized for mass production of banana. The process involves initiation of cultures from shoot tips obtained from the parent banana plant, shooting and rooting in the test tube, primary hardening in the laboratory, secondary hardening in the nursery and planting in the field. The advantages of tissue cultured banana are

- Disease free elite varieties ensured
- Rapid multiplication and early harvesting
- Uniform size and age of plants
- \* High quality fruit bunches
- • Availability throughout the year.



D.Singapore Red



Burana Gold



D.Black Cat



Emma White



D.de Fathima





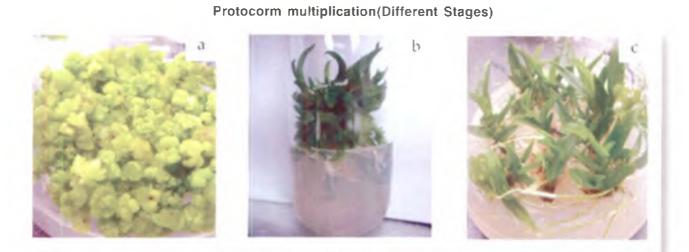




Tissue culture laboratory



Initiation of protocorms



plantlet regeneration



**Plantlet Rooting** 



**BANANA TISSUE CULTURE** 

Banana initiation Banana multiplication

**Banana Hardening** 

The generation of virus free planting materials is an ideal strategy to facilitate the movement of materials across the domestic and international boundaries. Tissue culture is a useful approach for generating virus free planting materials. The test for virus and quality will have direct impact on developing a vibrant tissue culture industry as the demand for certified tissue culture raised plants will grow exponentially.

The important virus diseases of banana are Banana Bunchy Top Virus (BBTV), Cucumber Mosaic Cucumovirus, Banana Bract Mosaic Poly Virus and Banana Streak Badna Virus. The banana viruses primarily spread through the use of infected suckers but secondary spread also occurs through aphid vectors. If virus free materials are not used as initial planting material, the field spread will be much faster. An annual loss of about 40 million rupees has been reported from Kerala state alone due to BBTV. The incidence of BBTV resulted an yield reduction of about 40% of bunch weight in cultivars like Nendran, Robusta, Red banana, Poovan etc.

In Kerala, at present most of tissue culture laboratories are under private sector. Several plants are produced annually through tissue culture techniques and distributed for large scale planting without testing the genetic fidelity. This will seriously affect the production of fruits in Kerala. Hence the production and distribution of virus indexed quality planting materials of banana to the farmers was started.

#### b. Need for micro propagation in orchids

Orchids are a group of attractive flowers having a wide range of diversity in their form, size, color and texture of flowers. This crop is very important in the cut flower industry of Kerala state. Its fascinating flowers have very good keeping quality and hence are of highest value as cut flowers. Some varieties (eg. Phalaenopsis) remain fresh for about three months in plants and for about a month if used as cut flower. Orchids are highly adapted to the agroclimatic conditions of Kerala. A humid warm atmosphere is congenial for most tropical orchids. They also require proper temperature for good growth and flowering.

It is a very slow growing crop and propagated vegetatively by 'keikis' (sympodial orchids) or stem cuttings (monopodial orchids). A maximum of 2-3 keikis are produced from a plant in a year. So the availability of the sufficient planting materials of the improved varieties is a serious problem and this limits the cultivation and spread of orchids. Cochin being a metro city, there is a great demand for its cultivation in homestead gardens as well as terraces of houses both as ornamental crop as well as for using as cut flower. One advantage of this crop is that a large number of plants can be accommodated in a small area and so its cultivation and marketing can be done in houses itself. No additional land is required and hence its cultivation and marketing as cut flower is most suited for Kerala condition.

At present tissue culture flasks are imported from Thailand, Singapore, Malayasia etc. by private entrepreneurs and the hardened plants are given to farmers at very high price. According to the size of plant and variety, the price varies from Rs. 100/- to Rs.750/- This high price and non availability of good quality planting materials limits its spread and large scale cultivation. Hence it is high time to undertake the mass production of varieties having greater demand and market value through micro propagation under public sector so that the seedlings can be made available to the farmers as per their requirement, at reasonable price.

The research centre could standardize efficient protocols for the mass multiplication of banana (Var. Nendran and Grand naine) and orchids and started the mass multiplication and distribution of quality seedlings of TC banana and orchids.

## THE SALIENT OUTCOME OF THE MAJOR EXTERNALLY FUNDED RESEARCH PROJECTS OPERATED AT THIS CENTRE

The Rice Research Station, Vyttila could be equipped well using the fund from the various research projects financed by different funding agencies. A financial outlay of more than six crores rupees could be brought to the station through various research projects funded by external agencies. The laboratory facilities, including both area and equipment, could be strengthened. As a result, a well equipped molecular breeding laboratory, an analytical laboratory, a Tissue Culture Laboratory and a biocontrol aboratory could be established in the centre. The important EAP projects operated at this centre are urnished below. FIVE DECADES OF GLORIOUS RESEARCH\_

SI.No.	Title of project	Name of P.I.	Outlay (lakhs)	Durat ion (y)	Funding Agency	Date of Start
1	Ad-hoc project on Rice - fish/ prawn components for an integrated farming system	Dr.N.K.Sasidharan Professor (Agron)	4.20	3		1998-99
2	Rice varietal improvement under abiotic stress	Dr.K.S. Shylaraj, Professor(PB & Gen)	3.63	3	KSCSTE, Govt. of Kerala	2003-04
3	<i>In vitro</i> and <i>in vivo</i> mutagenesis in rice and screening for tolerance to abiotic stresses	Dr.K.S. Shylaraj, Professor(PB & Gen)	21.60	4	BRNS, Govt. of India	2005 -06
4	Developing protocol for organic rice production in Pokkali tract (Phase I)	Dr.K.Anilakumar Professor (SS& Ag.Chem) & Head	25.00	3	RKVY, Govt. of India	2007-08
5	Boosting organic rice production of Kerala through Marker Assisted Selection and High quality seed production programme.	Dr.K.S. Shylaraj, Professor(PB & Gen)	60.15	3	RKVY, Govt. of India	2008-09
6	Maximising food production through on-farm develop- ment of an integrated farm- ing model for the coastal saline tract of Kerala	Dr. V. Sreekumaran Professor (Agron) & Head	32.90	3	RKVY, Govt. of India	2009-10
7	Developing protocol for organic rice production in Pokkali tract (Phase II)	Dr. Annie Koruth Assoc. Prof. (SS&AC)	25.00	2	RKVY, Govt. of India	2010-11
8	Soil Based Plant Nutrient Management Plan for AEZ in Kerala- Agronomy component	Dr.V. Sreekumaran Professor (Agron) & Head	3.55	2	State Planning Board	2010-11
9	Soil Based Plant Nutrient Management Plan for AEZ in Kerala- Soil Science component (Phase I)	Dr. Annie Koruth Assoc. Prof. (SS&AC)	7.37	2	State Planning Board	2010-11
10	Nutraceutical properties of the unique pokkali rice	Dr.K.S. Shylaraj Professor(PB & Gen)	10.54	1	RKVY (Paddy Mission)	2010-11
11	Strengthening rice seed production at RRS, Vyttila	Dr.V. Sreekumaran Prof.( Agron.) & Head	1.86	. 1	RKVY (Paddy Mission)	2010-11
12	Strengthening nursery faci- lities at RRS, Vyttila for the micro propagation of impo- rtant horticultural crops of existing tissue culture units	Professor(PB & Gen)	15.00	1	SHM	2010-11

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\_\_\_\_FIVE DECADES OF GLORIOUS RESEARCH

	adoption of Microbial inoculant technology for cropping system of Kerala.	-		<b>.</b>	Gok Plan	
	on the Development and	Professor & Head			Planning Board	
22	component - Phase II State plan network project	Dr.V.Sreekumaran	18.13	1	Gok Plan State	2013-14
21	Soil Based Nutrient Management Plan for AEZ in Kerala- Soil Science	Dr. Annie Koruth Assoc. Prof. (SS&AC)	17.00	1	State Planning Board	201 <b>3</b> -14
20	Seed and Nursery	Dr.V. Sreekumaran Professor & Head (Agron)	10.00	1	Plan fund, Govt. of Kerala	2013-14
19	Elucidation of salt tolerance mechanism and development of saline tolerant rice variety through Marker Assisted Backcrossing.	Dr.K.S. Shylaraj Professor (PB & Gen)	50.00	3	ICAR Govt. of India	2013-14
18	Commercial micro propagation of high demand high value crops as per National Certification System for Tissue Culture Plants	Dr.K.S. Shylaraj Professor (PB & Gen)	29.00	3	Plan fund, Govt. of Kerala	2012-13
17	Development of <i>Saltol Sub 1</i> mega rice varieties for flood prone salt affected areas and standardization of production technology	Dr.K.S. Shylaraj Professor (PB & Gen)	156.42	4	Kuttanad Devpt. XIII th Finance Commission, GOK	2012-13
16	Mericloning of Phalaenopsis and Vanda hybrids	Preeta Liz Korah, Pl Dr.K.S. Shylaraj, Prof. (Scientist Mentor)	14.80	3	KSCSTE, Govt. of Kerala	2011-12
15 <sub>.</sub>	Establishment of tissue culture facility for the mass production of virus free banana and orchid clones	Dr.K.S. Shylaraj Professor(PB & Gen)	70.00	1	DAC, Govt. of India	2011-12
14	Production of elite seeds and planting material	N.V. Satheesan Associate Professor (Ag. Ent)	4.20	1	GOK, Plan	2011-12
13	Studies on the native strains of beneficial micro flora in pokkali for bio- control and bio-fertiliser production	Dr.V. Sreekumaran Professor (Agron) & Head	20.00	1	GOK, Plan	2011-12

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The salient outcome of the various Externally Aided Projects operated at this centre are the following.

#### 1. ADHOC PROJECT ON RICE - FISH/ PRAWN COMPONENTS FOR AN INTEGRATED FARMING SYSTEM (1998- 2001)

Principal Investigator	: Dr.N.K.Sasidharan, Professor (Agronomy)
Financial outlay	: Rs.4.20 lakhs
Funding Agency	: ICAR

INNOVATIONS / TECHNOLOGIES DEVELOPED:

An ICAR ad-hoc project entitled" Rice-fish-prawn components for an integrated farming system for the coastal tract of Kerala" was implemented at the Rice Research Station, Vyttila, for three years from 20-1-1998. The main objective of the project was to evolve a sustainable integrated farming system with rice, fish and prawn as the major components. Six field experiments encompassing the various aspects of rice, fish/prawn integration was laid out during the project period. Growth analysis of rice-fish and prawn was done and yield data collected for three years. Field water samples, crop samples and soil samples were periodically collected and subjected to chemical analysis for their content of major and minor nutrients.

Compatibility of the saline tolerant tall pokkali rice cultivar, VTL-3, along with the semi tall culture- 1026 and Chettivirippu mutant with three fish species viz. pearl spot (*Etroplus suratensis*), rohu (*Labeo rohita*) and male population of tilapia (*Oreochromis mossambicus*) was tested for rice-fish dual culture. The rice varieties varied with respect to their yield, dry matter production, lodging and light infiltration to the lower canopy region. The new rice cultures 1026 and Chettivirippu mutant out yielded the traditional cultivar VTL-3 and proved their, sustainability for rice-fish dual culture. Among the fish species tested, pearl spot and rohu could not survive the harsh pokkali field conditions and were found unsuitable for rice - fish dual culture. All male population of male tilapia could grow along with rice and attain sufficient size at harvest in a period of six to seven months.

The impact of tides, which inundates and drain the pokkali fields was studied in detail to find out its effect on the soil productivity and sustainability of pokkali lands. The influence of tidal influx on pokkali soil and performance of rice varieties were studied in two separate field experiments laid out in adjacent fields of one enjoying tidal inundation and the other denied with it. The study revealed that the tidal influx increased the pH and dissolved ammonia of the field water. It also regulated the salinity, electrical conductivity and dissolved oxygen of the field water, which have a bearing on the flora and fauna.

The chemical properties of the pokkali soils also revealed to be very much influenced by the tidal action. The tidal action increased the pH, electrical conductivity and organic carbon content of the pokkali soils, which indicated the possibility of increasing the fertility of coastal tract in an inexpensive way. Differential response to rice varieties to tidal action could be observed during the initial year. However, the continued denial of tidal action adversely affected the growth and yield of rice varieties tested. It could also be realised that yield levels of more than 4 t / ha could be obtained from pokkali fields, which are exposed to tidal action, without the addition of bulky organic manure or costly inorganic fertilisers.

The biomass production during a single rice crop season from pokkali lands could be worked out to be 14 to 16 t/ha. In order to harness this photo synthetically fixed energy to economic products, utility of anionic ameliorants were tried for two seasons. The anionic ameliorants especially sulphur significantly influenced the grain yield during the first year while, the same could not exert any influence during the subsequent years. Application of other ameliorants viz. fine silica, lime and phosphorus alone and in combination though influenced the chemical composition of rice plant, didn't result in favourably altering the grain/

#### FIVE DECADES OF GLORIOUS RESEARCH

straw ratio of rice in pokkali fields, while the same was at par with the untreated control plot which gave the maximum grain yield. It could be inferred from the studies that lowering of Na / K ratio is the key for obtaining higher grain yields from pokkali soils. Interference in to the pokkali agro-ecosystem by way of addition of chemical inputs topples the Na/K ratio within the plant and adversely affects the grain yield.

Studies on simultaneous culture of the giant fresh water prawn, *Macrobrachium rosenbergii* along with the pokkali rice during the non-saline phase, did not yield any encouraging results. During both the seasons under trial, the giant fresh water prawn recorded total mortality due to the harsh grow out conditions.

Selective stocking of quality prawn species during the saline phase, without supplementary feeding was tried as an alternative to the traditional prawn filtration in pokkali fields. The suitability of white prawn (*Penaeus indicus*) and tiger prawn (*Penaeus monodon*) for rotational culture with rice, without any supplementary feeding could be well established. Short term culture of 60-80 days was proved to be desirable for interior pokkali fields, where the salinity levels are less.

A survey on bivalve molluscan resources available in the pokkali fields and adjoining backwater area revealed that the clams *Paphia malabarica* and *Villorita cyprinoids* are the two prominent species of the pokkali agro- ecosystem. Between the two species, *Villorita cyprinoids* found to be available in plenty and tried as a component of the pokkali integrated farming system. *Villorita cyprinoids* when grown in pokkali fields attained marketable size in 22 months. The study further revealed that though clams grow well in pokkali lands they are not suitable for simultaneous culture with rice, since the frequent field operations necessary for rice cultivation, adversely affect the growth and development of clams.

#### 2. RICE VARIETAL IMPROVEMENT UNDER ABIOTIC STRESS (2003 - 2006)

Principal Investigator : Dr.K.S.Shylaraj, Professor (Gen. & Pl.Br)

Financial outlay : Rs.3,63,000/-

Funding Agency : Kerala State Council for Science, Technology and Environment (KSCSTE)

#### INNOVATIONS / TECHNOLOGIES DEVELOPED

- Seven early duration rice cultures tolerant to salinity, acidity and submergence were selected for changing the rice prawn farming system into rice-prawn /fish- prawn farming system.
- Easy and economical methods of *in vitro* screening system for tolerance to salinity and acidity could be standardized.
- Anther culture plants could be induced through callus culture from the two indica rice genotypes.
- On farm demonstration of rice fish prawn farming system in the field revealed that male tilapia can be cultivated along with rice profitably and could get about 500 kg/ha tilapia which enhances the unit area income.
- It could also be demonstrated that tiger prawn can be cultivated along with rice in the low saline phase
  and enhance the unit area profit as Rs.1,30,000 /ha.

#### 3. *IN VITRO* AND *IN VIVO* MUTAGENESIS IN RICE AND SCREENING FOR TOLERANCE TO ABIOTIC STRESSES (2005-06 to 2008-09)

Principal Investigator	: Dr.K.S.Shylaraj, Professor (Gen. & Pl.Br)
Financial outlay	: Rs.21,00,000/-
Funding Agency	: Board of Research on Nuclear Sciences (BRNS), BARC, Govt. of India

FIVE DECADES OF GLORIOUS RESEARCH.

The envisaged objectives and the important outcome of the project were the following.

## 1. Induction of semi tall stature through in vitro and in vivo mutagenesis to pokkali rice genotypes retaining the tolerance to multiple abiotic stresses like salinity, acidity and submergence.

Pokkali and Cheruvirippu are the two widely cultivated land races of rice cultivated in the pokkali ecosystem. These two varieties are the chief saline tolerant gene donors of rice used even in the International Rice Research Institute, Manila, Philippines. Though gifted with complex tolerance to abiotic stresses such as salinity, acidity and submergence, these are tall lodging varieties having poor yield. But cultivation of these varieties without yield improvement made rice cultivation non-profitable in these areas. Hence high yielding tall varieties viz. VTL-3 and VTL-4 were evolved from this Centre and were the popular cultivated varieties of this ecosystem. But the farmers could realize only half the yield potential of these varieties, as about 40 - 50% yield loss is experienced by the lodging nature. Hence mutagenesis was attempted in these land races and tall high yielding varieties to induce semi dwarfism which in turn could improve yield, at the same time retaining their complex tolerance ability to the above mentioned abiotic stresses.

The seeds of Pokkali, Cheruvirippu, VTL-3 and VTL-4 were subjected to both gamma irradiation (5 KR to 60 KR) and chemical mutagen (EMS 0.2% to 2.0%).

#### Outcome of the study:

- Irradiation with gamma rays was found more effective than chemical mutagenesis for inducing semi dwarfism / dwarfism and earliness in rice.
- Gamma irradiation with 40 KR doze was effective in inducing semi dwarfism in pokkali rice genotype VTL-3. But semi dwarf mutants could not be induced with the same doze in case of the variety VTL-4. So the success of induction of mutants / correction of an agronomic trait is a chance in any mutation breeding programme.
- The semi tall mutants evolved from this study (four promising cultures) were recommended for farm trial and can be released for cultivation if found promising.

#### 2. In vitro callus induction and induced mutagenesis to induce semi dwarfism

In the pokkali ecosystem, as there is only one rice crop in a year, the breeding period required for the evolution of a high yielding saline tolerant rice variety takes a long period of 12 years. Hence it was felt as an urgent need to reduce the breeding cycle of varietal evolution. The only possible alternative is dihaploid breeding through anther culture technique which will yield homozygous dihaploid plants in the first generation itself. This will save 4 - 5 years of homozygocity advancement of breeding cycle in the conventional breeding method. This necessitated the need for standardization of anther culture technique in pokkali rice genotypes. These genotypes come under indica group which responds less for anther culture compared to japonica group. Hence a study was undertaken under the project to standardize the callus induction medium and regeneration medium of the pokkali rice genotypes so as to evolve saline tolerant high yielding rice varieties within a period of 5 - 6 years.

Two media viz. C medium and N6 medium could induce calli in pokkali rice genotypes. The callus induction % was highly influenced by the period of cold pre treatment. Panicles collected 3 - 4 days before emergence and stored at 4° C for 11 days was found to be ideal for callus induction. Further, the most ideal carbon source for better callus induction and callus proliferation was observed as maltose. Two hormonal combinations (2.0 NAA mg  $l^{-1}$  + 0.5 mg  $l^{-1}$  KTN and 2.0 mg  $l^{-1}$  2.4-D + 0.5 mg  $l^{-1}$  KTN ) of C medium and one hormonal combination of N6 medium (1.0 mg  $l^{-1}$  NAA + 0.5 mg  $l^{-1}$  KTN +2.0 mg  $l^{-1}$  2.4-D) induced callus. N6 medium with 1.0 mg  $l^{-1}$  NAA + 0.5 mg  $l^{-1}$  KTN induced direct morphogenesis. N6 medium



Mutation induced sterility Symptom in M1 Gamma Irradiation



Semi dwarf M2 plants in the field (VTL-3 40 KR)



Induced callus through rice anther culture



**Regenerated rice plant** 



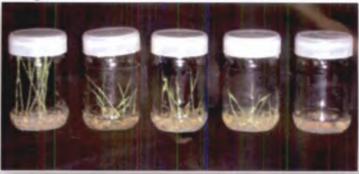
Hardened anther culture derived plant



Mutation induced anther sterlity

### In - vitro screening for abiotic stresses





Tolerance index: Survival % and visual salt injury (white leaf tip curling)

NS 4 dS/m 6dS/m 8 dS/m 10dS/m VARIETY JAYA



NS 4 dS/m 6 dS/m 8 dS/m 10dS/m VTL - 2



Seedlings after 10 days of acidity treatment





Root damage due to acidity

809353



#### \_FIVE DECADES OF GLORIOUS RESEARCH

supplemented with 1.0 mg I  $^{+}$  NAA + 0.5 mg I  $^{+}$  KTN was found most suitable for inducing morphogenesis and regeneration of more number of green plantlets. N6 medium with 1.0 NAA + 0.5 KTN + 2.0 2,4-D also could induce morphogenesis but most regenerants were observed as albinos. To reduce mortality and for better survival, the regenerated plants were transferred to liquid N6 medium (1/4 dilution) without hormones and maltose. After one week, these plants were transferred to small pots filled with paddy field clay.

*In vitro* mutagenic work was carried out by treating the rice callus with different low doses of Gamma - radiation (2KR, 4KR, 6KR, 8KR & 10KR). Also the combination treatment of both physical (Gamma-radiation-2KR, 4KR, 6KR, 8KR & 10KR) and chemical (EMS-0.5%,0.75%1%,1.5%,2%) mutagen was attempted.

This combination treatment was found to be more effective in VTL-3 rice cultivar than VTL-4 as drying and blackening of callus was less. This shows the differential response of varieties to the same dose of mutagen. But both these *in vitro* mutagenic treatments did not produce semi dwarf plants.

#### 3. The identification of biochemical markers for salinity tolerance screening

#### a. Improved salinity stress tolerance in rice by accumulation of osmoprotectant proline

Adverse environmental conditions such as drought and saline soils can reduce a variety of activities essential for respiration and photosynthesis in NaCl-sensitive plants. Unlike most toxins and herbicides, excess NaCl or insufficient water has no single cellular target. The deleterious effects of these stresses result from both dehydration, which can denature many proteins or membranes, and ion displacement, in which the accumulating chemical compound places inorganic cofactors needed for some enzymes to work efficiently. Plants exposed to these stresses induce many different types of genes. The expression of some may be merely symptomatic of the type of damage that ultimately leads to death.

Most high yielding rice cultivars give poor yield under saline conditions. Rapid accumulation of free proline in tissues of many plant species as a response to salt effect, drought or temperature stress, has been attributed to the protection of cellular membrane and enzyme stabilization and/or osmoregulation (Aspinall and Paleg 1981). Many workers have reported a several fold increase in the proline content under physiological and pathological stress conditions.

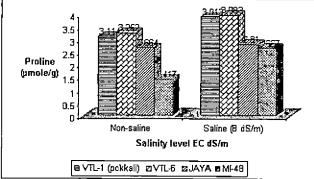
Hence the level of accumulation of proline in response to stress induced by NaCl was studied and explored the possibility of proline accumulation as a molecular marker for salinity tolerance screening.

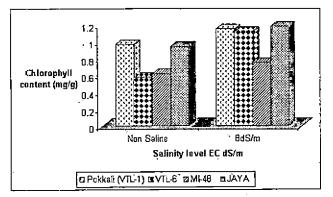
#### Outcome of the study

The increased root / shoot ratio under salinization appears to be an adaptation to salinity resulting in a more efficient water and nutrient uptake under saline stress. The proline accumulation was found increased under salinization both in tolerant and susceptible varieties of rice. At moderate level of stress, accumulation of proline provides necessary osmoregulation to the salt sensitive variety. On the other hand, the inherent high level of proline in salt tolerant variety, itself provides necessary osmotic protection at moderate level of stress. However, a sharp overproduction of proline is eventually required even for the salt tolerant variety to withstand higher level of stress justifying the credibility of proline as a selective marker for osmotic stress tolerance. However, these conclusions can be concreted only after a further study involving different saline concentrations and more tolerant varieties.

#### Proline accumulation under salinity stress in rice varieties of different tolerance level

#### Chlorophyll content (mg/g) of different rice varieties under saline stress





#### b. Antioxidant enzyme peroxidase in germinating rice seeds in relation to salt tolerance

It is well documented that abiotic stresses exert at least in part of their effects by causing oxidative damage (Smimoff, 1995). Oxidative damages are caused by reactive oxygen species (ROS) and excess amounts of ROS are harmful to many cellular components, including membrane lipids. ROS cause peroxidation of polyunsaturated fatty acids in the membranes (Smirnoff, 1995). Production of ROS is increased under saline conditions (Greenway and Munns, 1980; Hasegawa et al., 2000) and ROS-mediated membrane damage has been demonstrated to be a major cause of the cellular toxicity by salinity in rice, tomato and citrus. ROS are inevitable byproducts from the essential aerobic metabolisms, and they need to be maintained under sub lethal levels for normal plant growth. Hence, plants are equipped with an array of enzymatic and non-enzymatic antioxidant molecules to alleviate cellular damage caused by ROS. Multiple antioxidant enzyme systems are involved in the enzymatic scavenging of ROS. Superoxide dismutase react with the superoxide radical to produce  $H_2O_2$ . Hydrogen peroxide is scavenged by catalases and peroxidases.. Peroxidases are well known for their role in  $H_2O_2$  detoxification in plants.

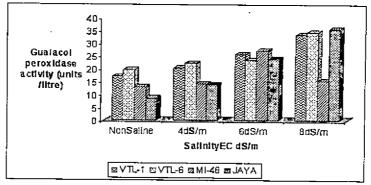
A large body of evidence has shown that the antioxidant enzyme systems are altered under abiotic stresses, including salinity. The quantitative and qualitative aspects of changes are often related to the levels of resistance to salinity. In rice, the salt-tolerant varieties have higher SOD activity and lower lipid peroxidation than the salt-sensitive varieties. Under this project an attempt was made to study the peroxidase enzyme level under salinization in rice varieties of different tolerance level.

Two saline tolerant varieties viz. pokkall and VTL-6, one moderately tolerant variety Jaya and a susceptible variety MI 48 were used for the enzyme activity studies. All the four varieties exhibited varietal difference with respect to chlorophyll content. The resistant variety pokkali has the maximum chlorophyll content followed by the moderately tolerant variety Jaya, susceptible variety MI-48 and the resistant variety VTL-6. But under saline condition (8 dS-m), the chlorophyll content was found increased in all the varieties. The increase in chlorophyll content might be due to a reduction in leaf tissue water content consequent to saline treatment. Wang and Nii (2000) also reported an increase in chlorophyll content on saline treatment of amaranthus plant. However, further studies are needed under different salinity levels to draw a valid conclusion.

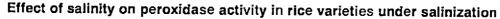
Chlorophyll content (mg/g	) of different rice varieties	under saline stress
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Salinity level	Chlorophyll content (mg/g)				
EC dS m <sup>-1</sup>	Pokkali (VTL-1) VTL-6		<u>MI-48</u>	JAYA	
Non Saline	0.963	0.573	0.615.	0.943	
8dSm-1	1.148	1.11 <u>3</u>	0.745	1.179	

The study revealed that varietal difference exists between varieties with respect to gualacol peroxidase activity under non-saline condition. The resistant variety VTL-6 has the maximum enzyme activity followed by pokkali and the susceptible variety MI-48.



#### Gualacol peroxidase activity under different salinity levels



EC (dSm <sup>-1</sup> ) Salinity	% increase of peroxidase activity over its non-saline check			
	VTL-1	VTL-6	JAYA	MI-48
4	20.43	13.08	5.24	1.26
6	51.27	18.35	176.22	14.14
8	96.67	75.46	314.22	2.55

Under normal condition the least enzyme activity was recorded by the variety Jaya (8.58 units I<sup>-1</sup>). As the salinity level increased, the peroxidase enzyme activity also increased up to 8 dS m<sup>-1</sup>. But in case of susceptible variety MI-48, the peroxidase activity was increased only up to 6 dSm<sup>-1</sup> and further increase in salinity caused a sudden decline in peroxidase activity. This clearly indicates that peroxidase activity can be taken as a criteria for assessing salinity tolerance level in seedling stage. In case of the moderately tolerant variety Jaya, there was two fold increase in peroxidase activity might be one of the major mechanisms operating in the variety Jaya. Hence it is conclusively proved that peroxidase activity increases with increase in salinity stress up to a particular level and thereafter it declines. As the peroxidase level of different varieties are different, the comparison of the level of increase of peroxidase should also be made with the peroxidase level of the same variety under non - saline condition. However, a further study with still higher level of salinity is needed to draw valid conclusions.

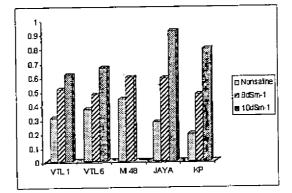
## c. Antioxidant enzyme superoxide dismutase (SOD) in germinating rice seeds in relation to salt tolerance.

Plants have developed various protective mechanisms to eliminate or reduce ROS, which are effective at different levels of stress-induced deterioration. The enzymatic antioxidant system is one of the protective mechanisms including superoxide dismutase, which can be found in various cell compartments and it catalyses the disproportion of two  $O_2$  radicals to  $H_2O_2$  and  $O_2$  The  $H_2O_2$  is eliminated by various antioxidant enzymes such as catalases and peroxidases which convert  $H_2O_2$  to water.

Moreover, ROS are inevitable byproducts of normal cell metabolism. But under normal conditions production and destruction of ROS is well regulated in cell metabolism. When a plant faces harsh conditions, ROS production will overcome scavenging systems and oxidative stress will burst. In these conditions, ROS attack vital biomolecules and disturb the cell metabolism and ultimately the cell causes its own death.

The result indicated that there was a negative relationship between SOD activity and lipid peroxidation or MDA content. The SOD activity increased with increasing salt stress levels. Under non saline condition, the SOD activity was maximum for the susceptible variety MI 48 and the high SOD activity of MI 48 was expressed at low salinity level of 8 dSm<sup>-1</sup>. At very high salinity level of 10 dSm, this variety did not express any SOD activity. The moderately tolerant variety Jaya expressed the maximum SOD activity at 10 dS/m which also indicate as a survival mechanism. The resistant varieties may express a still higher SOD activity above 10 dSm<sup>-1</sup> which was not tried in this study. When SOD activity was high, ROS, especially superoxide radical scavenging was done properly and thus, damage to membranes and oxidative stress decreased, leading to the increase of tolerance to oxidative stress. Salt stress increased the superoxide level in cells. If this radical is not scavenged by SOD, it disturbs vital biomolecules. The increase in SOD activity and

decrease in oxidative damage were closely related. Hence, the study indicated that SOD can be a biochemical marker for salinity tolerance screening.



## Superoxide dismutase activity under different salinity levels

#### Screening protocols against abiotic stresses

The coastal saline ecosystem has complex abiotic stresses like salinity, acidity and submergence. Hence the varieties evolved for this ecosystem should be screened against these stresses and efficient protocols need to be standardized for screening. In this study, we could standardize some dependable protocols for screening against these stresses.

Efficient protocols for *in vitro* screening for tolerances to salinity, submergence and acidity could be standardized under the project.

# 4. BOOSTING ORGANIC RICE PRODUCTION OF KERALA THROUGH MARKER ASSISTED SELECTION AND HIGH QUALITY SEED PRODUCTION PROGRAMME.

Principal Investigator	: Dr.K.S.Shylaraj, Professor (Gen. & Pl.Br)
Financial outlay	: 60.847 lakhs
Funding Agency	: Rakshtriya Krishi Vikas Yojana, (RKVY), Govt. of India

The low-lying areas of Kerala, the southernmost state of India, are potential areas of paddy production. But these areas are situated below the mean sea level and have serious problems of water-logging. About twenty five percent of total paddy lands in Kerala are water-logged; especially in Kuttanad, Pokkali, Kole and Kaipad areas. Among them Pokkali and Kole lands are the important water logged areas in the State. The lands for Pokkali cultivation are low lying marshes and swamps situated near the estuaries of streams and rivers not far from the sea. An integrated rice-shrimp/ prawn farming are practiced in these areas. One rice crop followed by shrimp/prawn capture provides a substantial subsidiary income to the farmer. The rice-shrimp rotational cropping has supplemented animal protein in the diets of the rural population. The system proved to be the most economic utilization of land, family labour, and also provides other benefits such as reducing insect pest pressure and natural regulation of the organic content of the soil. The major issues faced in these areas are mainly related to pollution, eutrophication, encroachment, reclamation, mining and biodiversity loss. As a result of pollution, draining, filling, etc. these water-logged areas have been under severe threat. The potential of these areas shave to be utilized rationally and necessary measures in conserving these areas need to be undertaken.

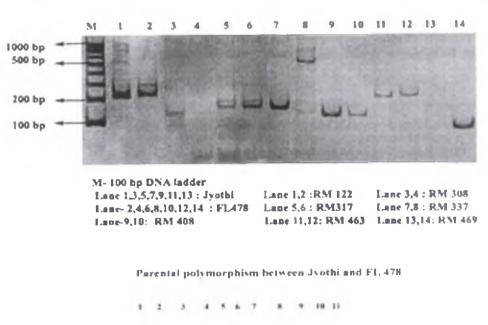
The system can be made sustainable only by integrating rice followed by prawn / fish cultivation. The rice cultivation in this ecosystem became difficult and non profitable due to non availability of labourers, high labour wages, climate induced changes, non availability of quality seeds of high yielding varieties, more potential saline tolerant varieties etc. To rejuvenate rice cultivation in this potential organic farming area with the medicinal / anti oxidant rich pokkali rice varieties, there is an urgent need to evolve saline tolerant high yielding varieties. Further, the salt intrusion into the major rice bowls of Kerala viz. Kuttanad, Kole lands etc. is a serious threat to our rice production. Hence the introgression of *Saltol QTL* of the Pokkali into the mega rice variety Jyothi through Marker Assisted Breeding was the major objective of this project.

#### The major achievements under the project are the following.

- The Saltol QTL of pokkali rice genotype could be introgressed into the most popular high yielding mega rice variety of Kerala viz., Jyothi through Marker Assisted Selection. Thirty two Saltol introgressed lines are being evaluated under field condition. After field evaluation, the most promising line/ lines can be released as Saltol Jyothi.for commercial cultivation in the saline agro ecosystem of Kerala.
- A total of twelve front line demonstrations were conducted in different padasekarams using the latest released semi tall high yielding varieties VTL-6, VTL-7 & VTL-8 (five demonstration plots during 2009-10 and seven demonstration plots during 2010-11). These demonstration plots helped to make the farmers convinced about the superiority of the semi tall high yielding varieties ( about double the yield compared to the tall high yielding varieties, easiness for harvest operation, and amenability for mechanized harvesting etc.) and thereby the adoption of semi tall high yielding varieties were increased and the coverage of high yielding varieties could be now increased above 80% and the yield could be doubled in 80% of the cultivated area in the Pokkali ecosystem (from 1.5 2.0 tons to 3.5 4.0 tons /ha) without the addition of any fertilizers or plant protection chemicals.
- During the kharif seasons of 2009-10 and 2010-11, a total of 58 tons of quality seeds of the semi tall non lodging high yielding rice variety VTL-6 could be produced by Farmer Participatory Seed Production Programme and the seeds were distributed to the respective padasekarams. Thus the seed requirement

Parental polymorphic assay of rice genotype using rice specific SSR markers

#### MARKER ASSISTED SELECTION THROUGH PARENTAL POLYMORPHISM ANALYSIS

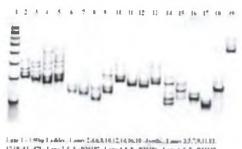




Lane 1. - 1000p Ladder, Lane 2.4,6.8.10.12,14,16.18. Jones 3.5,7,9,11,13. 17,19.43. d79. Lane 2.6.3 (N1413, Lane 4.6.5) (N1438, Lane 6.6.7) (N1459), Lane 8.6.9 (N1445, Lane 10.6.11) (N1447).

### b. SELECTION OF BACKGROUND MARKERS FOR RECURRENT PARENT SIMILARITY

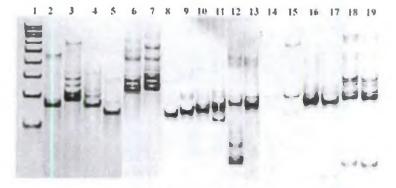
Parental polymorphism between Junihi and FL 478.



Lane L. 1999; Ladier, Lanes 2443; 1412; 1419; 1419; 1419; Janes 337; 1411; 1 45; 1441; 471; Lane 24; 31; 1991; 1; and 44; 7; 1991; 1; and 44; 7; 1992; Lane 84; 7; 0; 92; 1; and 17; 4; 11; 1992; 1; and 12; 4; 13; 1992; 1; and 14; 4; 14; 1992; 4; 1; and 19; 4; 11; 1992; 1; and 12; 4; 13; 1992; 1; and 14; 4; 14; 1992; 4; 1; and 19; 4; 17; 1992; 1; and 14; 19; 1992; 1; and 14;

#### **GENOTYPIC SCREENING OF BACKCROSSED POPULATIONS**

#### Parental polymorphism between Jyothi and FL 478



Lane I - 100bp Ladder, Lanes 2.4,6,8,10,12,14,16,18 -Jyothi, Ennes 3,5,7,9,11,13, 17,19 -FL-478, Lane 2 & 3 - RM17, Lane 4 & 5 - RM11, Lane 6 & 7 - RM122, Lane 8 & 9 - RM152, Lane 10 & 11 - RM154, Lane 12 & 13 - RM179, Lane 14 & 15 - RM184, Lane 16 & 17 - RM185, Lane 18 & 19 - RM 188.

#### SCREENING OF BCIFI PLANTS WITH FOREGROUND MARKER RMINM

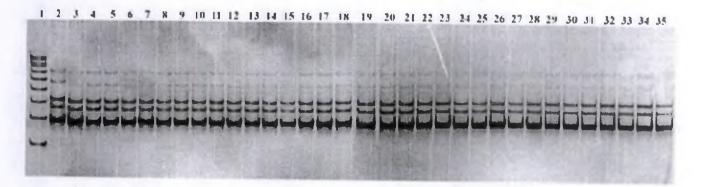
1 2 3 4 5 4 7 0 9 10 13 1	2 13 14 15 16 17 18 19 20 21 22 23 34 25 26 27 28 29 38 31 32 33 34 35 36	17 34 39 40 41
was an included if		ed and a

Lane 1- 100bp Ladder., Lane 2 - Jyothi, Lane 3 - FL-478., Lanes 4-41 - BCIF1 plants.

Screening of BC1F1 and BC2F2 progenies using 'saltol' marker RM8094.

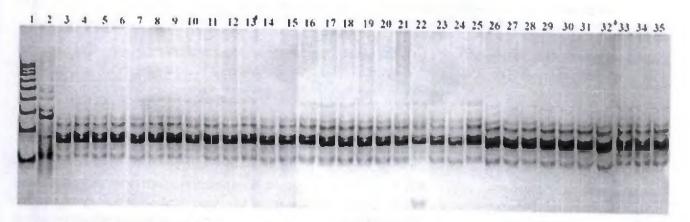
Lane 1 - 100bp Ladder, Lane 2 - Jyothia Lane 3 - FL-478, Lane 4&5 - BC2F1 progenies, Lane 6-35 BC2F2 progenies.

Screening of BC1F1 and BC2F2 progenies using 'saltol' marker RM493.



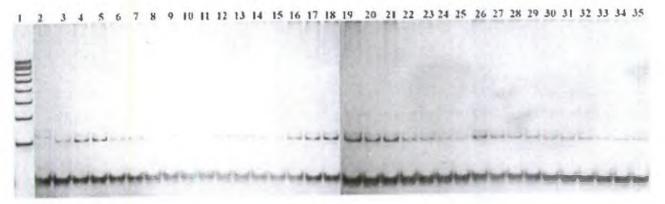
Lane 1 - 190bp Ladder, Lane 2 - Jyothi, Lane 3 - FL-178, Lane 4&5 - BC2F1 progenies, Lane 6-35 BC2F2 progenies,

## Screening of BC1F1 and BC2F2 progenies using 'saltol' marker SKC-10.



Lane 1 - 100bp Ladder, Lane 2 - Jyothi, Lane 3 - FL-478, Lane 4&5 - BC2F4 progenies, Lane 6-35 BC2F2 progenies.

## Screening of BC1F1 and BC2F2 progenies using 'Saltol' marker RM3412.



Lune 1 - 100bp Ladder, Lane 2 - Jyothi, Lane 3 - FL-478, Lane 4&5 - BC2F1 progenies, Lane 6-35 BC2F2 progenies.

for the cultivation of 250 ha of area during 2009-10 and 300 ha area during 2010-11 could be met under the project. Thus the project could enhance the area under high yielding variety and thereby the yield could be doubled through the production and distribution of quality seeds of saline tolerant high yielding varieties of rice, mainly VTL-6.

 Apart from this, the progressive farmers of the ecosystem were trained scientifically how to produce quality seeds with proper rouging, weeding, avoid mixing with off types etc.

There was marginal profit only when tall lodging HYV were cultivated in the pokkali padasekarams. When shifted to semi tall non lodging HYV, the farmers could realize the considerable profit of about Rs. 29,444/ - per ha. Further, if the cultivable pokkali area which are kept as fallow at present brings under cultivation, about 21000 tons pokkali rice can be produced which costs about41.65 crores rupees and the pokkali farmers can realize a total profit of about Rs. 20.65 crores from the whole pokkali area and can get a profit of about Rs.29,500/- per ha.

Being pure organic and medicinal rice, it is recommended to give premium price for pokkali and their value added products. Just like Njavara rice, the procurement price of Pokkali needs to be increased to a minimum of Rs.40/- per kg .lf so the income will increase further . If the paddy procurement price is enhanced to Rs.40/- or Rs.50/- per kg being speciality rice, the profit may increase to 2.36 times to 2.95 times.

On a broad perspective the project could lead to environment safety, sustainability, nutritional and livelihood security, employment and poverty alleviation.

### 5. MAXIMISING FOOD PRODUCTION THROUGH ON- FARM DEVELOPMENT OF AN INTEGRATED FARMING MODEL FORTHE COASTAL SALINE TRACT OF KERALA (2009-10 - 2012-13)

Principal Investigator	: Dr.V.Sreekumaran, Professor (Agron.)& Head
Financial outlay	: Rs. 32.90 lakhs
Funding Agency	: Rakshtriya Krishi Vikas Yojana,(RKVY), Govt. of India

This project was implemented to evolve a sustainable integrated farming model with rice, fish, prawn and crab as components for the coastal saline ecosystem of Kerala. A socio-economic survey of sample holdings and training programme on integrated farming practices for representative pokkali farmers also conducted under the project.

### RICE -CUM - FISH CULTURE (TILAPIA)





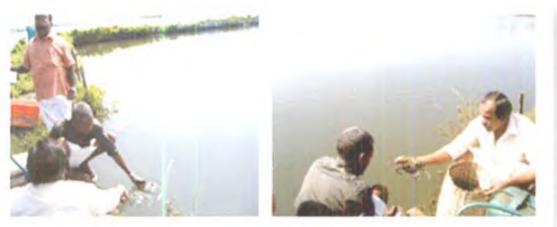


Tilapia fish harvest



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MUD CRAB CULTURE - CHELLANAM







Tiger prawn harvest



The survey showed wide knowledge gap on scientific farming practices being followed in the area. This offers much scope in improving the farming conditions through the adoption of good agricultural practices. As regards the rice cultivation, majority of the farmers used only traditional varieties namely, pokkali, chettivirippu etc., while very few only cultivated high yielding cultivars. The average yield obtained for majority of the (65 %) farmers ranged from 500 to 1000 kg / acre. Any yield below 1000 kg per acre was considered a loss sustained in rice cultivation in pokkali conditions, and thereby majority ended up in loss every year.

The results of the survey also revealed that majority (63 %) of the farmers preferred both prawn filtration and farming combined to either of them alone. But the standard pond preparation practices, a measure of scientific farming, was not being followed by majority of the farmers. Regarding the average yield of prawn obtained was considered, only 43 per cent of farmers produced an yield more than 200 kg / acre, while majority (57 %) came under less than 200 kg / acre category. This very low yield had resulted in loss to majority of the prawn farmers while taking into account the cost of cultivation under pokkali farming conditions.

The survey highlighted several constraints faced by farmers, of which the prevalence of viral disease of prawn, water pollution, high labour wages, non-availability of labour, finance, quality seed etc., among others were important. The training needs of farmers when assessed, it was found that majority lacked any formal training on improved cultural practices.

An one day farmers' meet and training programme on integrated farming was also conducted at the centre on 23-9-2010, in which about 200 farmers representing the pokkali tract, extension officers and scientists participated. It provided a good platform for the effective interaction between the participants and addressed their genuine problems confronting the pokkali farming. Availability of quality paddy seed, appropriate machinery for land preparation and harvesting, deepening drainage channels, premium price package including marketing for pokkali branded rice, providing threshing/processing yard, availability of seed, feed and technological inputs for shrimp/crab culture and introduction of crop insurance were the major constraints.

As a part of the development of a sustainable integrated farming model for the tract, several crop and aquatic animal modules were tried on station and on- farm conditions. The various modules tried included, raising a high yielding semi-tall rice variety VTL-8 during the low saline phase, simultaneous cropping of rice -cum- giant fresh water prawn (*Macrobrachium rosenbergii*) and rice -cum- tilapia fish (*Oreochromis mossambicus*) during low saline phase, selective culture of tiger prawn (*Penaeus monodon*) and crab in rotation during the high saline phase.

An experiment with a high yielding semi tall salt tolerant rice (VTL 8) was taken up during the low saline phase for three years from 2010 to 2012 in large plots following the typical system of traditional way of pokkali cultivation. A mean grain yield of 4203 kg / ha was obtained from the experiment without the addition of any fertilizers or chemical pesticides. Replacing the traditional tail varieties with a high yielding semi tall variety alone would improve the rice yield by 40 per cent and above.

The field experiment of semi-intensive culture of shrimp (*Penaeus monodon*) popularly known as tiger prawn was conducted in the centre during the high saline phase for three consecutive seasons. Standard pond preparation practices were carried out, and good quality disease free hatchery produced post larvae of the above species were stocked at a density of 40000 no's / ha. They were fed with commercial feed at the rate of 5 % of their biomass was given twice in a day. The hydro-graphic parameters such as salinity, pH, dissolved oxygen, temperature etc. were monitored at regular intervals. The salinity of water has never been found to be adequate for optimum growth. The third season culture was abandoned prematurely due to the severe mortality. The mean yield of 475 kg / ha of tiger prawn in a culture period of 89 days was

#### FIVE DECADES OF GLORIOUS RESEARCH\_

obtained under low to moderate salinity conditions. An on-farm front line demonstration of semi-intensive shrimp culture in four farmers' fields was also carried out with the same scientific cultural practices during the high saline phase of 2011 and a mean yield of 586 kg / ha was recorded during a culture period of 106 days under relatively good saline water conditions. As far as the rotational crop of tiger prawn during the high saline phase was considered, very high net returns / ha of Rs. 103300/- was obtained with a maximum benefit cost : ratio of 1.95. This system also generated an additional employment of 155 man days.

The giant fresh water prawn (*Macrobrachium rosenbergii*) was tried as a component crop in the rice -cum - prawn experiment on simultaneous culture during the low saline phase of 2010. The peripheral channels in the rice field were modified to accommodate the dual culture. The post larvae of the fresh water prawn were stocked after the sowing of the rice crop. The rice crop was harvested at maturity, while the prawn did not survive the harsh soil water conditions of pokkali which resulted in the complete mortality, implying little possibility of dual culture of fresh water prawn along with rice in similar growing conditions.

In an experiment of dual culture of rice and tilapia fish during the low saline phase of 2011, it was found that both rice and fish fared well with an average rice yield of 4690 kg / ha was obtained, while an average yield of tilapia fish of 670 kg / ha recorded from the simultaneous culture. The net returns / ha from simultaneous culture of rice and tilapia fish was found to be Rs. 44800/- which was 114 % more than when rice alone was considered, which implies that inclusion of an additional component crop of tilapia along with rice is an economically viable practice for enhancing the profitability of the system. The success of the system also depends on selecting relatively bigger sized juveniles of fish seed considering the very short cropping period of 3 months. This rice -fish integration was also found to generate 35 man days more than the rice alone system.

A high value crab culture was carried out as a field trial in a farmer's field in the pokkali area during the high saline phase of 2011. Bigger sized juvenile crabs were stocked under semi-intensive farming with supplementary feeding. An average yield of 405 kg / ha was obtained during a culture period of nearly 6 months. The maximum weight attained was 1.05 kg for a single crab which fetched more than Rs. 700 /- per kg in the export market. The semi-intensive culture of crab recorded the highest net income of Rs. 145500/- per ha, but with a slightly lower benefit : cost ratio of 1.76 due to the high cost of cultivation. Though the net income obtained was very high, the crab cultivation was not found to be very popular among the farmers. The availability of large sized seed crab, cannibalism of crab species, high cost of supplementary feeding, loan dependence of export market are the main constraints.

In the integrated farming system, it was observed that the rice-fish/prawn system in the Pokkali lands continuously replenished the nutrients which in turn helped to increase the yield of rice and fish substantially. Thus, rice-fish/ prawn integration could not only make rice production attractive by increasing the productivity and profitability but also render it more organic and environment friendly.

Thus, it can be concluded that raising a high yielding semi-tall rice variety VTL-8 during the low saline phase and semi-intensive selective culture of tiger prawn *(Penaeus monodon)* during the high saline phase in rotation was found to be the best integrated farming model for the coastal saline ecosystem in terms of economic viability and sustainability.

#### 6. DEVELOPMENT PROTOCOL FOR ORGANIC RICE PRODUCTION (DPORP) IN POKKALI TRACTS

Principal Investigator : Dr.K.Anilakumar, Prof. & Head & Dr.Annie Koruth, Assoc. Professor (SS&Ag.Chem)

Financial outlay : Rs. 50.00 lakhs

Funding Agency :Rakshtriva Krishi Vikas Yojana, (RKVY), Govt. o India

The pokkali paddy, which to an extent is saline resistant, flood resistant and acid-resistant, is the wonder

crop cultivated in the pokkali lands. It is cultivated by organic methods without any fertilizer application in the marshy and waterlogged tracts which lie near the mouth of the rivers and are close to the sea. Thirty padasekharams in the pokkali tract were monitored and assessed the major, secondary and micro nutrient status.

#### Soil characteristics of Pokkali tract

- The pokkali soils, are highly acidic with 87 % of the soils exhibiting acidic nature of which 81 % of the soils having pH < 5.5 with extremely acidic to strongly acidic reaction. Under highly acidic conditions of less than pH 5.5 there is high solubility of AI resulting in its toxicity condition and consequent Mg deficiency in all the soil samples. In soil samples having pH more than 5.5 there was no solubility of AI and no toxic effect and in addition there was availability of Mg.</p>
- The pokkali tract which is comprised of low lying waterlogged areas along the saline coastal belt of Kerala is under the influence of tide, the salinity of these soils, EC > 4 dSm<sup>-1</sup> exists in 34 % of the samples
- \* 94 % of the soils are having medium to high organic carbon status.
- In the pokkali lands 60 % of the samples are having high available P status and 32 % medium status. Thus P application is not recommended.
- With 68% high K status and 13 % medium K status the potassium fertilizer application is not recommended.
- The secondary nutrient Ca is adequate in 79% of the soils, 100% deficiency of Mg and 100% sufficiency of S is characterized in the pokkali tract.
- \* The micronutrients Zn, Cu and B are 100% adequate.
- During the high saline phase there was no hazardous chemicals /heavy metals in the water source except for Nickel which was more than 0.2 mg L<sup>-1</sup> the tolerance limit for irrigation water, while the concentration of Lead, Arsenic, Boron, Aluminium, Zinc, Copper, Iron and Manganese was less than the tolerance limit for irrigation
- The pesticide residues of organo phosphorous, organo chlorine and synthetic pyrethroid compounds were not detected in the water and soil samples during both high and low saline phase. The detection limit being 0.5 μg L<sup>-1</sup>.
- The soil and plant nutrient status of 30 padasekharams in the pokkali tract were monitored which indicated the highly fertile soils very suitable for organic rice production.
- Only little response to added biofertilizers due to the high fertility status of the soil and huge numbers of natural soil borne microorganisms.
- The native flora is sufficient to solubilize the insoluble P in soil. The application of K solubilizers are not required due the high K status of soil. P-solubilizing organisms did not survive in the pokkali soils which are acidic. Native flora of azospirillum and P solubilizers were present in the pokkali soil while native K solubilizers are absent in pokkali soil.
- The biofertilizers either to supplement N or P or K or combined had no significant effect on grain yield in the pokkali soils owing to the high fertility status of the soil which has a capacity to produce 3.6 t/ha without the addition of any nutrients both in the form of biological and chemical.
- \* The application of inorganic fertilizers with or without lime do not have any positive influence on grain yield of pokkali varieties.

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- The chemical fertilizers had little effect on rice yield in pokkali tract. The pokkali fields are highly suited for organic farming. There is percentage yield reduction due to the application of chemical fertilizers in the Pokkali soil. Thus the fertilizer recommendation of 20:40:0 kg N: P2O5and K2O ha-1 for Pokkali rice was withdrawn from the package of practices recommendations, Kerala Agricultural University, based on the response studies conducted at this centre. This has helped to declare the entire Pokkali tract as fully "organic zone".
- For a sustainable rice production in the pokkali tract, there should not be any hindrance for the natural tidal inflow of water. This enriches the soil, without the application of any chemical fertilizers enabling the production of the renowned organic pokkali rice and sustaining the pokkali eco-system.

#### Protocol for organic rice production for pokkali tracts

- 1. Liming to correct pH to >5.5
- Adopt soil testing to ensure the soil fertility status of soil.
- 3. Proper water management procedures to be followed for balanced availability of nutrients to the crop and removal of toxic elements.
- 4. Do not hinder the natural inflow and outflow of tidal currents.
- 5. Chemical fertilizers or any other chemical ameliorants to be avoided.

All the pokkali padasekharams monitored are very highly fertile and suitable for organic rice production. The balanced availability of nutrients to crop to be ensured. Based on the results of the project, it is possible to award/ issue "Organic Certification" to padasekharams, so as to facilitate farmers to earn premium prices for pokkali products.

## 6. Soil Based Plant Nutrient Management Plan for Agro Ecosystems of Kerala

(Soil Science Component)

Principal Investigator	: Dr. Annie Koruth, Assoc. Professor (SS&AC)
Financial outlay	: 7.37 lakhs
Funding Agency	: State Planning Board

#### Soil Fertility of Ernakulam District

Surface soil fertility was assessed through collection of composite samples from farmer's fields all over the district. Around 150 to 250 soil samples were drawn from farmer's fields in each Panchayat of the district and representing different crop production systems. Total of 8,875 samples were collected and analysed for 13 soil fertility parameters: soil reaction, soluble salts, major plant nutrients (Nitrogen, Phosphorus and Potassium), secondary nutrients (Calcium, Magnesium and Sulphur) and micro-nutrients (Iron, Manganese, Copper, Zinc and Boron). The data sets generated were primarily used for assessing soil fertility, plant nutrient input advisories to the farmers and plant nutrient management plans for Panchayats, blocks, agro-ecological units and for the district. This section presents an analysis of the data and their interpretations. The content of soluble salts in the soils was negligible and no deficiency of micro-nutrients iron and manganese was recorded in any of the analysed samples. Hence analysis and interpretation of the data pertaining to the said parameters are not included.

#### 1. Soil Reaction

Acidification of soils, a serious constraint to crop production, has assumed serious proportion in the district with eighty eight per cent of the samples testing for acid reaction (Fig.1). Fifty two per cent of the samples tested for extremely to strongly acid reaction (pH <5.5). The primary cause for development strong acid condition in soils of the district is the inherent acidity of the soils, without regular application

of lime to neutralize acidity generated. Application of liming materials based on soil test results is essential to improve crop productivity. In the absence of soil test results, liming at the rate 600 kg per hectare is recommended.

In the agro ecological units of the district, Pokkali lands had only 28% of the samples testing for extremely acid to strongly acid reaction, while 48% of the samples in the southern and central foot hills and 72% of the samples from southern high hills tested for strong soil acidity. When the data sets were analysed for samples drawn from specific crop production systems, 40 per cent of the samples from coconut system tested for strong soil acidity. It was followed by samples from fields of banana (50%), rice paddies (78%) and rubber plantations (78%).

#### 2. Plant available Nitrogen in soils

Estimate of plant available nitrogen in soils through determination of organic carbon content showed fairly satisfactory levels in the district. Only around 20 % of the samples were deficient in available nitrogen. In the Pokkali lands 42% of the samples had low N status while in the rest of the areas it was less than 13%. Samples from different cropping systems also showed similar trend, less than a quarter of samples testing low levels. Application of nitrogenous fertilizers to crops according to soil test is recommended. In the absence of soil test results follow the doses prescribed for a crop in the Package of Practices Recommendations Crops of the Kerala Agricultural University.

The coastal sandy soils have low content of organic matter and bases, low water and nutrient retention. It is essential to maintain high levels of organic matter in these soils. High levels of organic matter not only provides part of the nitrogen requirement of crop plants, but also enhance nutrient and water retention capacity of soils and create favourable chemical and biological environment.

#### 3. Plant available Phosphorus in soils

Plant available phosphorus was deficient only in 6 per cent (Fig.3) of the soil samples drawn and tested from the district. The high proportion of soil samples tested for excess levels of phosphorus 84%, suggest considerable build up of the nutrient, consequent to high input of the fertilizers containing phosphorus. The result of the study points to the possibility for reduction in the use of the costly phosphatic fertilizers. Again, correction of soil acidity through liming can lead to release of phosphorus fixed by soil constituents into the available pool. Hence it is recommended to get the soils tested regularly and apply fertilizers accordingly. In the absence of soil test results apply only fifty per cent of the dose fixed for crops. The data sets on available phosphorus did not reveal significant variation among samples tested for high levels of available phosphorus, with more than 70% samples testing for very high to extremely high levels. It is highly essential to reduce phosphatic fertilizer inputs in the light of the test results. High levels of the nutrient can impair the uptake of many other essential nutrients by plants.

#### 4. Plant available Potassium in soils

Only 21% of the samples tested for deficient levels of potassium (Fig.4). Almost the same trend was noticed for soil samples drawn from different agro-ecological units and cropping systems, except for samples from banana, rice and rubber production system wherein 31% of the samples were deficient in the nutrient.

The results of the study points to the need for regular application of potassium fertilizers in deficient areas in as many splits as feasible, in doses recommended for crops by the Package of Practices of Kerala Agricultural University. Maintenance of high levels of organic matter and abating soil acidity through liming can go a long way in regulating potassium nutrition of the plants in these soils.

#### 5. Plant available Calcium in soils

The deficiency of available calcium was observed in nearly 50% of the soils (Fig.5). No significat deviationswere observed when soil samples from different agro ecological unit and cropping systems were separately analysed, except for samples from rubber growing-areas, wherein almost 64% of the samples recorded deficient levels of calcium. Application of liming materials to alleviate soil acidity shall ensure the supply of the nutrient wherever deficient.

#### 6. Plant available Magnesium in soils

Magnesium, an essential secondary nutrient is deficient in 61% (Fig.6) of the composite soil samples drawn and tested. Soil samples drawn and tested for available magnesium from different AEUs and cropping systems showed significant difference. Magnesium deficiency was more pronounced in the pokkali lands, 85% and in the coconut areas, 73%; rice areas, 69% and rubber growing areas, 66%. It is necessary to apply magnesium sulphate regularly in accordance with soil test results or MgSO4 at the rate of 80 kg per hectare where soil test results are not available.

#### 7. Plant available Sulphur in soils

Sulphur deficiency was observed only in about a quarter of the soils in the district (Fig.7). Most phosphatic fertilizers contain sulphur as an additional constituent and that is responsible for fairly satisfactory levels in soil, despite the low retention capacity of the soil for sulphur. Significant variation was observed among the different agro-ecological units and cropping systems, the AEU 12- Southern and Central Foot Hills and in the rubber plantations, 41% of the samples drawn and tested recorded low levels of the nutrient. Application magnesium sulphate can take care of sulphur requirement of crops.

#### 8. Plant available Zinc in soils

Zinc is a micro-nutrient required only in very small quantities by plants. The deficiency of zinc is negligible in the soils of Ernakulam (Fig.8). The element often occurs as a contaminant in phosphatic fertilizers, including rock phosphate. High input of phosphatic fertilizers might have ensured adequate level of zinc in soils. The micro-nutrient zinc need to be applied only on the basis of soil test results indicating deficiency.

#### 9. Plant available Copper in soils

Copper an important micro-nutrient is adequate to an extent of 89% in the samples tested (Fig 9). The same pattern follows in the different cropping systems. Copper is an important ingredient of common fungicides used to combat many fungal born diseases of crop plants in humid areas. Copper input from fungicides and the retention of the element in soil organic matter may explain the adequate levels of the copper in soils. The micro-nutrient copper need to be applied only on the basis of soil test results in areas indicating deficiency.

#### 10. Plant available Boron in soils

Boron is another essential micro-nutrient required only in very small quantities by plants. The deficiency of boron is a major problem, its intensity extending to 78% of the samples tested (Fig.10). There is variation in the extent of deficiency for soil from different agro ecological units and also crop production systems. Deficiency of the nutrient was comparatively more in soils of southern and central foot hill regions (86%), rubber growing areas (87%), banana (83%) and in the rice areas (78%). In the absence of soil test results apply 10 kg of borax per hectare. Foliar application 0.2 per cent solution of borax is better.

### Recommendations on soil fertility management for Ernakulm District

1. Management of soil acidity is of paramount importance in the extremely to strongly acidic soils with

pH<5.5 which is prevalent in more than 50% of the crop production systems of banana, rice and rubber in the district. Liming of acid soils in accordance with soil test results is highly essential. In the absence of soil test apply lime at the rate of 600 kg/ha.

- 2. Regular application of organic matter or recycling organic matter is essential to maintain favourable chemical environment in the low activity clay soils and retention of applied plant nutrients.
- 3. Apply nitrogenous fertilizers in accordance with soil test results or as recommended in the Package of Practices Recommendations Crops of Kerala Agricultural University.
- 4. It is highly essential to reduce the costly phosphatic fertilizer inputs in the areas with very high to extremely high levels of P. In the absence of soil test results reduce the dose to the tune of fifty per cent of the recommended dose.
- 5. There is the need for regular application of potassium fertilizers in deficient areas in as many splits as feasible, in doses recommended for crops by the Package of Practices Recommendations Crops of Kerala Agricultural University.
- 6. Application of liming materials to alleviate soil acidity shall ensure the supply of deficient calcium.
- 7. Apply magnesium sulphate @ 80 kg/ha to ensure adequate levels of magnesium and sulphur to crops.
- 8. The micro-nutrients zinc and copper need to be applied only on the basis of soil test results indicating deficiency.
- 9. The deficiency of boron is a major problem and in the absence of soil test results apply 10 kg of borax per hectare.

#### Conclusions

Analysis of the data pertaining to land utilization in the district over the last five decades pointed to substantial increase in land put to non-agricultural use and consequent reduction in the net area sown and gross cropped area.

The area under rice declined to ten per cent of the original with most low lands being filled and leveled for construction of buildings or crops other than rice. At present rice in the district is mainly confined to coastal low lands and pokkali lands, where conversion of rice lands to other crop production systems is practically impossible.

There was considerable increase in area and production for banana and other plantains, but the productivity increase was only 7.2 to 8.7 tonnes per ha over the last five decades indicating the need for soil fertility management to increase the productivity of the crop. The area under tapioca reduced by 65% but production doubled and productivity of tapioca witnessed more than five fold increase during the period under consideration. Rubber area expanded by 73% with 36 fold increase in production.

Rubber productivity increased to 1580 kg/ha, almost approaching the state average. The area under coconut is remaining almost steady now .The production has reduced, pointing to the need for proper management of this important crop for a sustainable coconut production system. The per palm productivity of coconut reduced from 38 nuts, in early sixties, to 31 nuts in 2011. Pepper area reduced by 65% resulting in drastic reduction in production, four fold. The productivity declined to half. Productivity of cashew declined from 1.6 to 0.24 tonnes per ha in the last five decades as a result of 90% reduction the cropped area. There was increase in pineapple area and reduction in the area under ginger, mango and the area under sugarcane vanished by 2008-09.

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Surface soil samples collected and analysed from the district indicated extensive acidification with eighty five per cent samples testing for acid reaction and of this fifty two per cent of the samples tested for extremely acid to strongly acid reaction (pH <5.5) requiring application of liming materials to improve crop productivity.

The organic matter status showed fairly satisfactory levels providing adequate mineralisable nitrogen in the district. The deficiency of available nitrogen is seen only in about quarter of the area. The available phosphorus status is high in almost all soil samples with more than 70% samples testing for very high to extremely high levels, indicating the need to reduce the application phosphatic fertilizer. High levels of the nutrient can impair the uptake of many other essential nutrients by plants. Available potassium was deficient in twenty one per cent of the samples, the remaining with medium to high level of the nutrient.

The deficiency of available calcium is a major problem in nearly 50% of the soils where the soil reaction is extremely acid to strongly acid in reaction. Among the secondary nutrients, deficiency of calcium was noted in 50% and magnesium in 61% of the samples while sulphur only in a quarter of the samples.

Surface soils were adequate in micro-nutrients iron and manganese with negligible deficiency for zinc and copper. The deficiency of boron is a major problem and its intensity is extending to 78% of the samples tested.

## 7. STUDIES ON THE NATIVE STRAINS OF BENEFICIAL MICRO FLORA IN POKKALI FOR BIOCONTROL AND BIOFERTILSER PRODUCTION (2012-13)

Principal Investigator	:	Dr.V.Sreekumaran, Professor (Agron.)& Head
Financial outlay	:	Rs. 20.00 lakhs
Funding Agency	;	GOK Plan project

Pokkali soils are inherently fertile and productive due to the active presence of micro flora and fauna native to this ecosystem. Preliminary studies indicated that there is much scope for the isolation of acid tolerant strains of several bacteria, blue green algae and other fungi specific to Pokkali region and its immense potential for mass production and utilization in organic agriculture. The direct influence of Pseudomonas on plant growth is mediated either by release of auxin like substances or through improved uptake of nutrients in the environment. The indirect promotion of plant growth is achieved when fluorescent Pseudomonas decreases or prevents the deleterious influence of phyto pathogens by secreting sideropores with a high affinity for Fe 3 – thus creating aritificial scarcity of iron for phyto pathogens. So it can be treated as both biocontrol and biofertilizer agent. P solubilizers help in the transformation of insoluble phosphate into soluble form that can be easily absorbed by the plants and thus promote the plant growth

Soil samples were collected from 36 locations in the pokkali tract. Rhizosphere soil sample from healthy plants were taken for isolation. The microflora available in these soil samples were isolated and identified for further utilization.

Preliminary studies on the available microflora of pokkali tract revealed that there is abundance of micro flora including Pseudomonas sp. P solubilising fungus and other N fixing bacteria which are acid tolerant strains useful for commercial exploitation on purification and further evaluation of its efficacy on crops.

A biocontrol laboratory with fermentor (100 L capacity), autoclave. Laminar airflow cabinet, microscopes, diesel power generator (62.5 KVA) etc. could be established and mass scale production and distribution of Pseudomonas fluorescence could be initiated. A revolving fund scheme for the continuous production of the biofertilizers and biocontrol agents on regular basis has been started functioning.

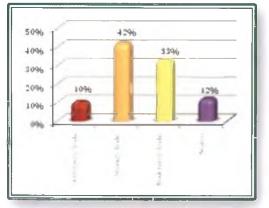


Fig. 1 Frequency of Soil reaction (pH) classes

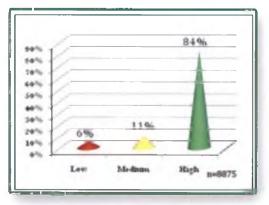


Fig. 3 Frequency of Available Phosphorous classes

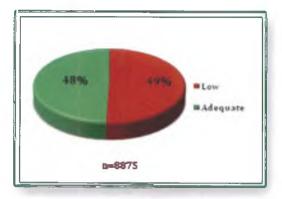


Fig. 5 Frequency of Available Calcium classes

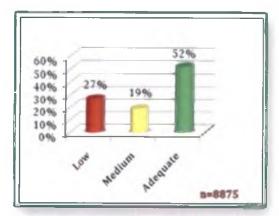
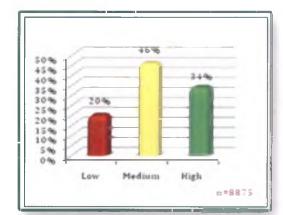
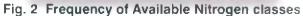


Fig. 8 Frequency of Available Zinc classes





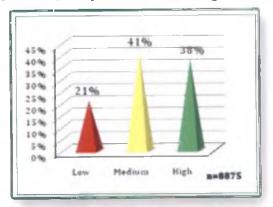


Fig. 4 Frequency of Available Potassium classes

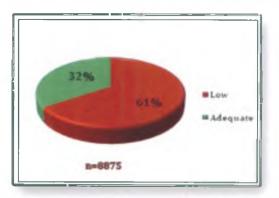


Fig. 6 Frequency of Available Magnesium classes

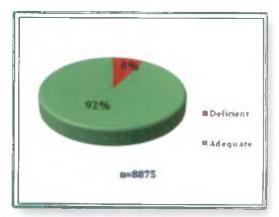
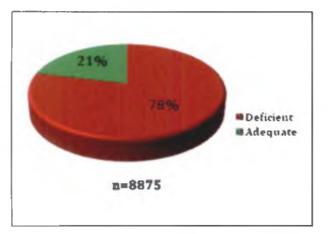


Fig. 7 Frequency of Available Sulphur classes





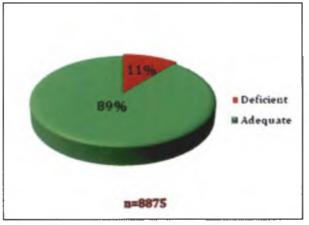
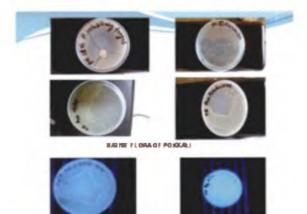


Fig.10 Frequency of Available Boron classes





**BIO CONTROL LABORATORY** 



## Protein and Nucleic Acid Separation and visualization Unit



POLYACRYLAMIDE GEL ELECTROPHORESIS UNIT



**GELOCUMENTATION UNIT** 



AGAROSE GELELECTROPHORESIS UNIT



UV TRANS-ILLUMINATOR



SPECTROPHOTOMETER



**REFRIGERATED CENTRIFUGE** 



COOLING CENTRIFUGE



MINI SPIN

## Instruments used for Molecular Biology Works

### Instruments used for Molecular Biology Works



PCR THERMAL CYCLER



MINIPOR THERMAL CYCLER



PCR THERMAL CYCLER



ELISA UNIT

## **Analytical Instruments**



ATOMIC ABSORPTION SPECTROPHOTROMETER



**KELPLUS NITROGEN ESTIMATION SYSTEM** 



FLAME PHOTOMETER



MICROWAVE ACCELERATED REACTION SYSTEM

#### INFRA STRUCTURAL FACILITIES DEVELOPED THROUGH EXTERNALLY AIDED PROJECTS

The laboratory facilities including lab building and sophisticated instruments could be developed using the fund received from the above mentioned projects. An exclusive tissue culture laboratory ( 3000 sq. ft area) could be commissioned for the mass production of virus indexed quality tissue culture plants of banana, orchids and other ornamentals like anthurium, zyngonium etc. The strengthening of the existing laboratories could also be done.

An excellent molecular breeding laboratory could be established using the funds from the RKVY project and Ecorestoration project (XIII Finance Commission). With the present facility, it is possible to introgress both abiotic and biotic stress tolerances into the mega rice varieties and remodel them for cultivation in the stress situations.

A well equipped analytical laboratory with sophisticated instruments could be established using the funds from the RKVY project with facility for analysis of major, secondary and micro elements. The regular analysis of soil, plant and water samples of various research centres of KAU, Department of Agriculture, other institutions and the samples of farming community on a subsidized rate is being done at this centre. Thus, apart from research, service and income generation activity could be done using the laboratory facilities developed. A good facility including fermenter, autoclave, laminar air flow, hot air oven, mixing chambers etc. for the large scale production of bio control agents / bio fertilizers could be established.

The out lab facilities like hybridization house, green house structures, hardening structures, orchid houses etc. could also be developed using the project fund. Further, necessary farm implements could also be procured using RKVY fund.

The scientists of Rice Research Station, Vyttila, technical and supporting staff acknowledge our profuse thanks to the various funding agencies for the support rendered to this centre for its all round development.

#### CURRENT SCENARIO OF RESEARCH

The centre has now advanced its research into biotechnology at molecular level and started the molecular breeding of rice varieties to introgress the various abiotic stress tolerance genes / QTLs into the mega rice varieties so as to make it suitable for the cultivation of rice in the adverse ecosystem of Kerala. Futher, the centre could develop efficient economic protocols for the mass propagation of different banana varieties and orchid varieties. Apart from mass propagation, programmes for the production of novel hybrids of orchids (Dendrobium, Phalaenopsis and Vanda) are being carried out successfully.

The formulation of soil fertility cards (padasekaramwise), development of cost effective and profitable integrated modules for rice / prawn -fish - crab, culture isolation and selection of suitable soil microbes for the formulation of bio fertilizers / pesticides etc are being carried out. The important ongoing major research projects are the following.

## 1. Development of Saltol Sub1 mega rice varieties for flood prone salt affected areas and standardization of production technology

Principal Investigator	: Dr.K.S.Shylaraj, Professor (Gen. & Pl.Br)
Financial outlay	: 156.42 lakhs
Funding Agency	: XIII Finance Commission, Govt. of India

The impact of global warming-induced sea level rise due to thermal expansion of near- surface ocean water has great significance to India due to its extensive low-lying densely populated coastal zone. Sea level rise is likely to result in loss of land due to submergence of coastal areas, inland extension of saline intrusion and ground water contamination and may have wide economic, cultural and ecological

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#### FIVE DECADES OF GLORIOUS RESEARCH\_

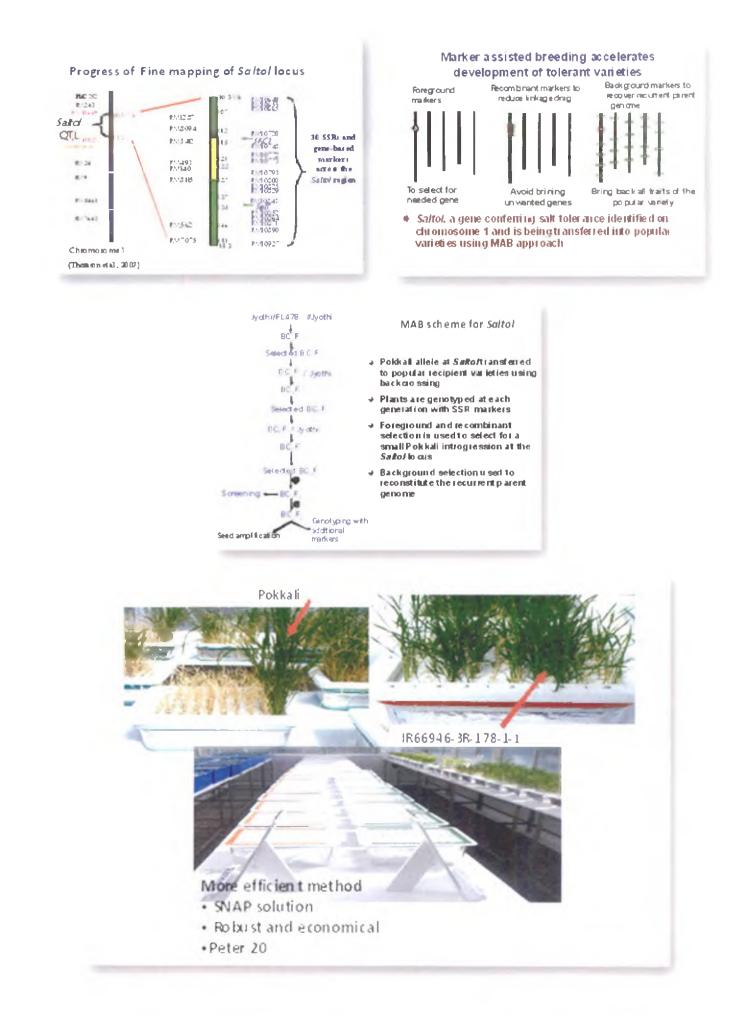
repercussions. Observations suggest that the sea level has risen at a rate of 2.5 mm year -1 along the Indian coastline since 1950s. A mean sea level rise of between 15 and 38 cm is projected by the mid 21st century along India's coast. Added to this, a 15% projected increase in intensity of tropical cyclones would significantly enhance the vulnerability of populations living in cyclone prone coastal regions of India. Apart from field salinity due to salt water intrusion, the frequent occurrence of floods and resulting crop loss has become a curse to the paddy fields in the coastal areas. The salinity intrusion to Kuttanad, the 'Rice Bowl of Kerala' was prevented by Thanneermukkom bund . But due to the leakage through this bund and due to sea level rise, salinity intrusion has become a problem in Kuttanad ecosystem also. So salinity and flood have become additional problems in Kuttanad apart from severe incidence of pests and diseases. The HYVs developed and used in Kuttanad do not possess the ability to withstand salinity and submergence (for two to three weeks according to the severity of the floods.) Hence, to sustain the rice cultivation in the paddy fields of Kuttanad ecosystem, Kari lands and salt affected coastal regions both in Alappuzha and Ernakulam districts, there is an immediate need to breed rice varieties possessing resistance /tolerance to salinity and submergence for a period of up to two to three weeks.

Rice (*Oryza sativa*) is sensitive to salinity, which affects one fifth of irrigated land worldwide. Progress in salinity tolerance breeding is slow due to the limited knowledge of the genetics of tolerance, complexity of the several tolerance mechanisms involved , inadequate screening techniques, low selection efficiency and poor understanding of salinity and environmental interactions. Through recent developments in molecular marker analysis , it is now feasible to analyse both the simply inherited as well as quantitative traits and then identify individual genes controlling the trait of interest. Molecular markers could be used to tag quantitative trait loci (QTL) and to evaluate their contributions to the phenotype by selecting for favourable alleles to these loci through Marker Assisted Selection scheme aiming to accelerate genetic advance. Advanced backcross QTL analysis can be used to evaluate mapped donor introgression in the genetic background of an elite recurrent parent.

There exists tremendous variation for salt tolerance within varieties in rice, providing opportunities to improve crop salt-stress tolerance through genetic means. Some attempts to develop salt-tolerant genotypes were based on highly tolerant traditional rice cultivars i.e. Pokkali and Nona-Bokra. (Gregorio and Senadhira, 1993). A major QTL located on chromosome 1 was identified for salt tolerance using F8 recombinant inbred lines (RILs) of Pokkali/ IR29 cross (Gregorio et al., 1997). This QTL governed the Na+. K<sup>+</sup> uptake ratio and accounted for 64.3 to 80.2% of the phenotypic variation in salt tolerance. This chromosome 1 segment was further saturated using RFLP and SSR markers using the RILs (Bonilla et al., 2002). The identified QTLs for Na<sup>+</sup>, K<sup>+</sup> and Na<sup>+</sup>/K<sup>+</sup> uptake ratio accounted for 39.2, 43.9 and 43.2% of the phenotypic variation. This segment of the chromosome 1 was further fine mapped by using near isogenic lines (NILs) of IR29 using Pokkali as the donor with microsatellite markers (Niones, 2004). It should be mentioned that Pokkali has been the most widely used salt tolerant parent by rice breeders. A newly developed line FL478 derived from a cross between IR29 and Pokkali was used as a novel source of salinity tolerance at seedling stage (Walia et al., 2005). Eight QTLs were found responsible for the variation in their K<sup>+</sup> and Na<sup>+</sup> content, among which SKC1distinguished as a major QTL for the K<sup>+</sup> and Na<sup>+</sup> shoot content and was mapped on chromosome 1, using F2 populations derived from a cross between Niponbare and Koshihikari cultivars (Ren et al., 2005).

The development of DNA / molecular markers have irreversibly changed the disciplines of plant genetics and plant breeding. The most promising application of these markers in breeding is for cultivar development and is called Marker Assisted Selection (MAS). MAS refers to the use of DNA markers that are tightly linked to target loci to assist phenotypic screening. By determining the allele of a DNA marker , plants that possess particular genes or quantitative trait loci (QTLs) may be identified based on their genotype rather than their phenotype.

MAS may greatly influence the efficiency and effectiveness for breeding compared to conventional breeding.

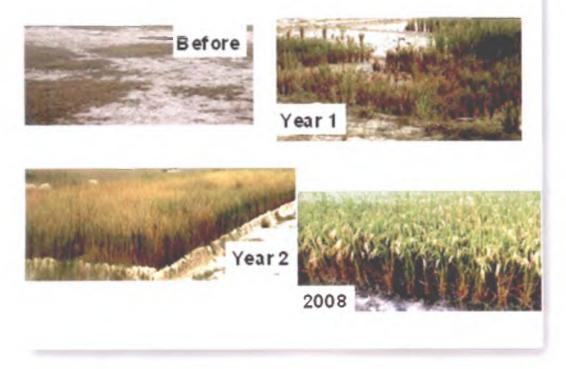


## New salt tolerant lines developed at IRRI

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iR.	6 1 9	2.0	-3 8 -2 2 - 2 - 1	Wagwag
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IR	6.5.5	5.6	-A C 1 .2 .5 .4	ICCP 266.2.49.28.3
			-4 B -4 -3	FCCP266-2-49-28-3



## Tolerant varieties impact in farmers' fields



#### \_FIVE DECADES OF GLORIOUS RESEARCH

The fundamental advantages of MAS compared to the conventional phenotypic selection in breeding are 1) greater efficiency or accelerated line development in breeding programs and the selection based on molecular markers may be more reliable since it avoids influence of environmental factors on field trials. Moreover the 'background markers' may also be used to accelerate the recovery of recurrent parents during Marker Assisted Backcrossing.

In Kerala, the changing scenario in climate is bringing more and more coastal areas under abiotic stresses like salinity, submergence, acidity etc. The saline coastal areas of Kerala which limits rice cultivation with reduced yield were located in Kari lands of Alleppy, Pokkali lands of Ernakulam, Mala areas of Trissur and Kaipad areas of Kannur districts. But at present, due to the climatic changes and global warming the northern Kuttanad areas and some parts of Kole lands have also become saline. The normal high yielding varieties can not be cultivated in these areas and for successful rice production in these salinity affected areas the development of saline tolerant HYV of rice is very essential. The introgression of *Saltol QTL* into the already existing promising varieties like Uma, Jyothi, Jaya, Aiswarya etc. through MAS is the most easy way. As Kuttanad is situated below sea level and due to untimely rainfall and poor drainage, field flooding is also a severe problem in these areas. So the flood tolerant gene *Sub1* also needs to be incorporated in these varieties. The present project at the Rice Research Station, Vyttila envisages the incorporation of both *Saltol QTL* and *Sub1 QTL* to our promising HYV of rice like Uma, Jyothi, Jaya, Aiswarya etc. so as to make them suitable for cultivation in these areas and to yield better in the stress situations. Pokkali is the source of *Saltol* gene and FR 13 A is the source of *Sub1* gene.

#### The programme of introgression of abiotic stresses

Hybridization between the recurrent parents (female) - Jaya, Jyothy and Uma each with donor male parents FL 478 (Salinity tolerant) and Swarna Sub1 (Submergence tolerant)

Parental polymorphism survey for recurrent parents with molecular markers: foreground, recombinant and background SSR markers (Jaya, Jyothy, Uma, FL-478 and Swarna Sub1)

Phenotyping and genotyping of F1 progeny for true hybridity and abiotic stress tolerance (salinity & submergence tolerance)

BC1F1 production (crossing Selected F1s with respective recurrent parents)

Genotyping of BC1F1s with polymorphic markers.

Evaluation of BC1F1 progenies involving foreground, background and recombinant selection for the target genes and recurrent parent genotype.

Production of BC2F1 (Selected BC1F1 X Respective recurrent parent)

Evaluation of BC2F1 progenies (with polymorphic SSR markers)

Production of BC2F2 (Selfing of selected BC2F1).

Hybridization of selected BC2F2 with Saltol gene and BC2F2 with Sub1 gene to produce megavariety

Evaluation of F1 mega variety progeny- genotyping and phenotyping

Production of F2 seeds (Selfing of selected F1s)

Phenotypic and genotypic screening of F2 progeny for true hybridity

Field testing - CYT and MLT will be done as part of KAU project and the abiotic stress tolerant lines can be released for cultivation to the farmers.

# Progress of works under the project:

### A. Molecular Works:

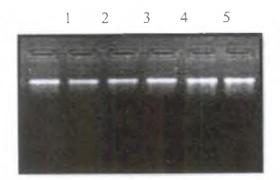
a. Primer Designing: The works regarding foreground, recombinant and background SSR markers along with trait specific candidate gene designing as well as selection from the already published database http: //www.gramene, http: //rgp.dna.affrc.go.jp/cgi-bin etc have completed. The nucleotide sequences of the genes of interest were obtained from the GenBank database at NCBI and were used to design specific primers using primer3 software.

**b.** Primer optimization: Primers serve as initiation sites for the addition of bases by Taq DNA Polymerase. For successful PCR, primers must be added in molar excess over the amount of target DNA. Here we optimized the primer volume as  $10\mu$ M, working stock from which  $1\mu$ I is taken per  $20\mu$ I PCR reaction, ie; in each PCR reactions  $0.5\mu$ M forward and reverse primers are taking part. Based on the melting temperature (Tm) of the primers of interest, the optimization of the annealing temperature of each primer was done. The annealing temperature used for a particular primer/template combination is usually optimal at plus or minus 7°C of the primer Tm. The standardization is done with the help of gradient PCR protocols.

**c.Genomic DNA isolation**: One month old leaves of the healthy plants were collected and immediately genomic DNA isolated to avoid degradation. The isolation process was done by following CTAB method (IRRI). The isolated genomic DNA was purified using RNase and PCI (Phenol : Chloroform : Isoamyl alcohol). Extracted DNA was checked through Agarose Gel.

d. Polymerase Chain Reaction: Parental Screening Using SSR markers: The genomic DNA of elite rice varieties Jyothi, Jaya and Uma (recurrent parents) along with a salt tolerant variety FL 478 and a submergence tolerant rice variety Swarna Sub1 were extracted and parental polymorphic assay using 240SSR rice specific background markers was completed.

# Plate 1 a : DNA isolated from Recurrent and Donor parents (Submergance tolerance)

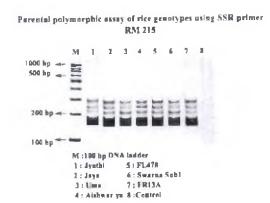


	ane	1:	Jyothi
			Jaya
			Uma
L	ane	4:	Aiswarya
L	ane	5:	Swarna Sub1
L	ane	6;	FR13 A
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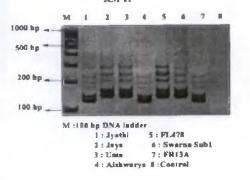
Plate 1b: DNA isolated from Recurrent and Donor parents (Salinity tolerance)



Lane 1: Jyothi	
Lane 2: Jaya	
Lane 3: Uma	
Lane 4: Aiswarya	
Lane 5: FL-478	
Lane 6 : FL-478	

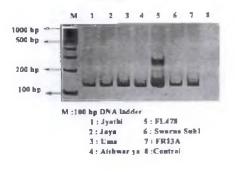


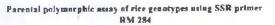
Parental polymorphic assay of rice genotypes using SSR primer RM 11

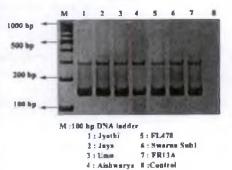


Parental polymorphic assay of rice genotypes using SSR primer RM E 7 18 м 1 2 3 a. 5 6 1000 hp 500 bp 200 bp -100 bp ----M :100 by DNA Indder 5 : FL478 1 : Jyathi 1: Jaya 6 : Swaran Subl 7 : FR13A 3 : Uma 4 : Aishwar yn 8 :Control

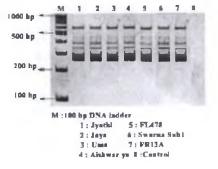
Parental polymorphic assay of rice genotypes using SSR primer RM 510



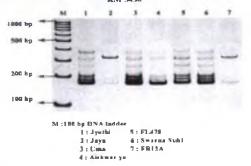




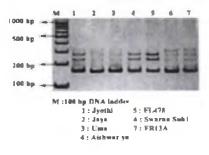
Parental polymorphic assay of rice genotypes using SSR primer RM 431

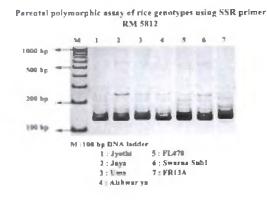


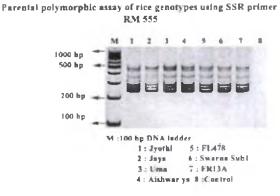
Parental polymorphic assay of rice genetypes using SSR primer 28 M \$430



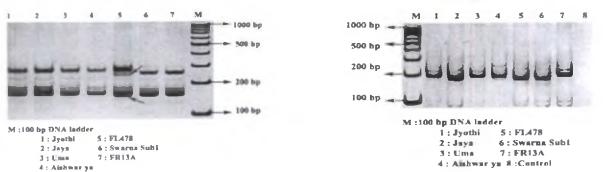
Parental polymorphic assay of rice genetypes using SSR primer RM 7382







Parental polymorphic assay of rice genotypes using SSR primer RM 6 Parental polymorphic assay of rice genotypes using SSR primer RM 129



## Plate 2: Molecular profiling of parental genomes for background selection

# Foreground and recombinant marker selection for salinity and suvmergence tolerance.

Genotyping using the 30 salinity tolerant primers and 44 submergence tolerant primers were completed.

#### **B**. Breeding works:

- 1. Hybridization between the recurrent parents (female) Jaya, Jyothi and Uma each with donor male parents FL 478 (Salinity tolerant) and Swarna Sub1 (Submergence tolerant) were successfully done.
- Phenotyping assays for salinity and submergence tolerance were done with parents of interest under study.
- Healthy mature F1 seeds were harvested and dried under the sun for one week and stored under - 20°C.
- 4. Molecular primers have been designed and selected from public database. These primers were synthesized (sigma) and are optimized for molecular works.
- 5. Parental genotyping using SSR background markers were started and are in progress. The background selection using SSR background markers is doing to identify background introgression. The microsatellite markers that revealed fixed (homozygous) alleles at non- target loci at one generation were not screened at the next generation.

The anticipated introgression work is expected to complete within two years and after field evaluation, the introgressed lines can be released for cultivation in the problem areas.

# II. Elucidation of salt tolerance mechanism and development of saline tolerant rice variety through a Marker Assisted Backcrossing.

Principal Investigator	: Dr.K.S.Shylaraj, Professor (Gen. & Pl.Br)
Financial outlay	: 50.00 lakhs
Funding Agency	: ICAR, Govt. of India

Rice (Oryza sativa) is sensitive to salinity, which affects one fifth of irrigated land worldwide. Progress in salinity tolerance breeding is slow due to the limited knowledge of the genetics of tolerance, complexity of the several tolerance mechanisms involved, inadequate screening techniques, low selection efficiency and poor understanding of salinity and environmental interactions. So a clear understanding of salinity tolerance mechanism operating in different land races need to be explored so as to pyramid these mechanisms in a single variety. Pyramiding of different salinity tolerance mechanisms in a single variety with extra tolerance to salinity.

Further identification and validation of biochemical markers for salinity tolerance is of prime importance for screening programme. There is very good chance of the presence of alternate sources of salinity tolerance in the saline tolerant Pokkali land races and the identification of such sources will help for the pyramiding of different QTLs in one genetic background and thereby making it more tolerant than the available varieties.

The germplasm at the Rice Research Station, Vyttila is a treasure of saline tolerant genes and there is greater scope to get alternate sources of salinity. Hence the development of a RIL population for searching.... the new source of salinity is also aimed at in this project.

Observational studies conducted at the Rice Research Station had revealed that different mechanisms of salinity tolerance are operating in the two land races of the germplasm (Shylaraj et al. 1995). Hence elucidation of the ionic mechanism of salinity tolerance is the major thrust envisaged in this project. The studies conducted in the BRNS Scheme at the Rice Research Station, Vyttila has revealed that there is great scope for validating some of the enzymatic and non enzymatic biochemical markers for salinity screening programme (Shylaraj et al. 2006 & 2008).

The envisaged programme in brief under the project include

- 1. Seed collection of different Pokkali land races: Pokkali, Chettiviruppu, Cheruvirippu, Karuthakuruka, Ponkuruka that are saline tolerant; and Jyothi, saline sensitive
  - Ť
- 2. Seedling stage (hydroponic) and reproductive stage screening of the tolerant varieties and susceptible variety in two different salinity levels of EC-8, EC-12 and control nutrient solution
- 3. Morphological characterization of the saline tolerant and susceptible varieties (Root length, Shoot length, Fresh weight, Dry Weight, Stress Tolerance Index and Tissue Tolerance Index)
- 4. Study of the ionic mechanism: Macro nutrient and Micronutrient analysis of Na, K, P, Zn, Ca and Mg of the salinity tolerant and susceptible varieties
- 5. Anatomical studies of the root and stem of salinity tolerant and susceptible varieties
- Identification of biochemical mechanism/markers, both enzymatic and non-enzymatic, involved in salinity tolerance

7. Raising of Recombinant Inbred Lines (RILs) using resistant land races like Cheruviruppu, Chettiviruppu, Karuthakuruka and Ponkuruka; and the susceptible line, Jyothi

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- 8. Molecular characterization of the RILs using markers
- 9. Identification of the alternate sources of salinity tolerance: Parental polymorphism study of selected parents, susceptible and check variety using known markers

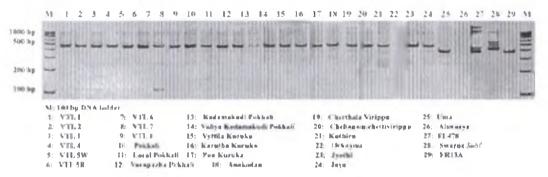
#### 10. Plot dendrogram using UPGMA software

#### The progress of work under the project:

The project has initiated only recently and the important research results are summarized below.

## Molecular analysis of Salinity tolerance in selected rice varieties

Recombinant screening: Genotyping of rice varieties using SSR locus specific primer AP 3206

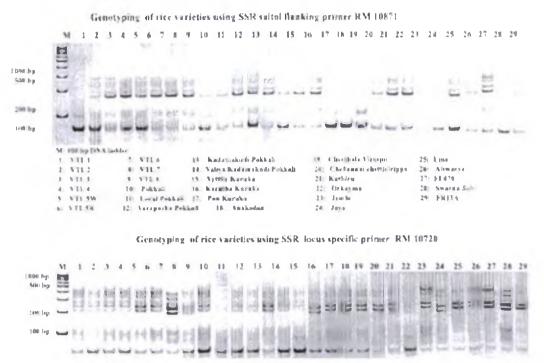


#### Canotyping of rice varieties ming SSR sailed flanking primer RSI 496

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#### Genotyping of rice varieties using SSR locus specific primer RM 493

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2:	VIL2	JE VILT	141	Andreas Maria and a knowl Polyden b	20:	Chellanam chettistrippe	26:	Airmarya
- Ja	VTL 3	St. VILa	15:	VyrBli Kuzuka	21:	Kuthlen	27:	FE-478
- 40	\$ 11.4	Die Politali	16:	Karatha Karaka	22:	f De Brans man	28:	Second 205
4.	VTL 5W	11: Logal Paidabl	17:	Pon Karadan	2.5:	J yu (h)	29:	FRIES
60	VIL58	11: Varapatha Pat	deali	18: Annahodan	34	Jana		

Genotyping of rice varieties using SSR local specific primer RM 10793



#### Foreground screening:

Genomic screening with salinity tolerance markers

Primer AP 3206	Type of SSR marker	Inference
AP 3206		
AI 0200	Recombinant	Polymorphic
RM 8094	Recombinant	Polymorphic
RM 3412	Recombinant	Polymorphic
RM 493	Foreground	Polymorphic
RM 490	Foreground	Polymorphic
RM 10696	Foreground	Polymorphic
RM 10720	Foreground	Polymorphic
RM 10748	Foreground	Polymorphic
RM 10772	Foreground	Polymorphic
RM 10793	Foreground	Polymorphic
RM 10825	Foreground	Polymorphic
	RM 8094 RM 3412 RM 493 RM 490 RM 10696 RM 10720 RM 10748 RM 10772 RM 10793	RM 8094RecombinantRM 3412RecombinantRM 493ForegroundRM 490ForegroundRM 10696ForegroundRM 10720ForegroundRM 10748ForegroundRM 10772ForegroundRM 10793Foreground

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12	RM 10871	Foreground	Polymorphic
13	RM 10713	Foreground	Polymorphic
14	SKC 10	Foreground	Polymorphic

Scoring of resulted banding patterns is in progress and thereby the genetic relationships can be characterized.

## III. Mericloning of Phalaenopsis and Vanda Orchids

Principal Investigator : Mrs. Preeta Liz Korah

Scientist Mentor : Dr.K.S.Shylaraj, Professor (Gen. & Pl.Br)

Financial outlay : 14.80 lakhs

Funding Agency : KSCSTE, Govt. of Kerala

**ORCHIDS**, the most beautiful flowers in God's creation, comprise a unique group of plants.Taxonomically, they represent the most highly evolved family among monocotyledons with 600-800 genera and 25,000-35,000 species. Many species of Dendrobium, Phalaenopsis and Vanda are renowned for their very showy and attractive flowers and their great ornamental value. The most important hybrid in trade internationally is Phalaenopsis Blume (over 22,000 man-made hybrids).

Phalaenopsis and Vanda orchids have high economic value in the floriculture industry. These hybrids are becoming prominent in Kerala both for ornamental and cut flower purpose. The non-availability of good quality/true to type planting material at reasonable price is the most important limiting factor for its spread and cultivation at a large scale. At present these hybrids are imported from Singapore, Malaysia, Thailand etc. by middle men and grown in private nurseries and distributed to consumers at an exorbitant price. The import cost and the profit realized by the middle men enhances the sale price of these hybrids. This limits its fast spread and its large scale cultivation is made unaffordable for the common man due to high initial investment and hinders the development of orchid industry in Kerala. The present project has been formulated as a solution to this problem.

In vitro propagation makes it possible to clonally mass propagate hybrids of commercial value and to conserve rare and endangered species. However, *in vitro* culture technologies are still a challenge in Phalaenopsis and Vanda hybrids because of the slow growth of plantlets, low multiplication rate, poor rooting and somaclonal variation. Although seed-raised plants can be used for conservation and breeding for the selection of superior features; genetic characteristics including seasonality, inflorescence, flower color and type are not uniform. In this regard, micropropagation through mericloning is an important strategy in obtaining genetically stable plants and improvement of quality. However, not all genotypes of Phalaenopsis and Vanda respond to the same protocol under the same culture conditions.

The project envisages at developing a cost effective, rapid and efficient propagation method for the identified hybrids on a commercial scale through mericloning via the culture of protocorm like bodies from meristems or direct regeneration of multiple shoots. The most adaptable and demanded hybrids will be collected through homestead survey.

#### Cost effective protocols developed from the project

- A highly cost effective protocol was standardized by substituting sodium hypochlorite(4% w/v) @ Rs.250/l. by local commercial bleach (20%v/v) @ Rs.15/l. There was no significant difference in explant survival percentage.
- As the source of carbon source in the medium, commercially available sugar could be used successfully which could reduce about 90% cost of sucrose.
- The bacteriological grade agar could be substituted by industrial grade agar which could reduce about 50% cost of agar in tissue culture.

## Outcome of the project

- The tender flower stalks (2-3 opened flowers) bearing buds and shoot tip explants were identified as the best explants for meristem culture in phalaenopsis.
- Half MS medium with BA and IAA concentration was found ideal for multiple shoot initiation in Phalaenopsis.
- Varietal variation with respect to response to culture was also observed.
- In Vanda hybrids, good direct multiple shooting (12-15) was observed from leaf bases of axenic seedlings in half-strength MS medium with Thidiazuron (TDZ) & CW in 1.5 month old culture.

# Phalaenopsis Hybrids



Dtps..Myastical Dream



**Dtps** .Leopard Prince



Dtps .Joy Angel Voice



Dtps.Sogo Melody



Phal.Sogo Lit Angel

Vanda Hybrids



Phal.Everspring Angel



**VDoctor Anek** 



**VPat Delight** 



**VPure Wax** 



VRobert's Delight



Vlokyo Blue



VSansai Blue



Multiple shooting in Phalaenopsis



Regenerated plantlet



Vanda leaf base



Multiple shooting



Green House

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## The Officers in charge of the Research Centre

- 1. Prof. T.F.Kuriakose, Professor
- 2. Prof. A.I.Thomas, Professor
- 3. Sri.P.K.Vijayan, Research Officer
- 4. Sri.K.K.Jayadevan, Research Officer
- 5. Dr.P.J.Joy, Professor
- 6. Sri.George Peter, Research Officer
- 7. Prof. T.U.George, Professor
- 8. Prof. P.J.Tomy, Professor
- 9. Prof. K.M.George, Professor
- 10. Prof. K.Chandrasekharan Nair, Professor
- 11. Dr.E.Tajuddin, Professor
- 12. Dr.Sivan Pillai, Professor
- 13. Dr.C.G.Rajendran, Professor
- 14. Dr.K.Anilakumar, Professor
- 15. Dr. V.Sreekumaran, Professor

## Other activities of the Research Centre

#### 1. Production of Bio-fertilizer / Bio-control agents

Soil contains useful micro organisms of bacterial, fungal and algal origin which helps plants to absorb nutrients. For sustainable crop production efficient strains of these microorganisms can be artificially cultured in the laboratory and incorporated into soils either directly or through seeds. The cultured micro organisms packed in some carrier material like peat, lignite powder for easy application in the field are called bio-fertilizers. They accelerate certain microbial processes in the rhizosphere which augment the extent of availability of nutrients in a form easily assimilated by plants.

Bio-fertilizers have important role in integrated nutrient management. They are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable crop production.

The different biofertilisers include Azospinillum, azotobacter etc. and biocontrol agents like trichoderma Plant Growth, Promoting Rhizobacteria *Pseudomonas fluorescens* etc. Rhyzobium strains are used in legumes like pulses, groundnut, soybean. It can fix 50-200 kg N/ha and can increase yield up to 35 percent. As a first step, the station has started the production and distribution of *Psudomonas fluorescence*.

A good facility including fermenters, autoclave and laminar air flow, hot air oven etc. for the large scale production of bio control agents / biofertilisers could also be established.

#### 2. Seed production and distribution

The regular production and distribution of good quality breeder seed and truthfully labeled seeds of VTL rice varieties and vegetable seeds like amaranthus, bhindi, cowpea, ash gourd etc. is also done at this station for the benefit of farming community

### 3. Production and distribution of hybrid coconut seedlings

Recently the centre has initiated the collection of hybrid seed nuts of released varieties for the production and distribution of hybrid coconut seedlings to the needy farmers.

#### 4. Training and classes to the Agricultural officers and farming community

The scientists of this station are offering training programmes and handling classes for both the extension officers and the farming community on various subjects.

#### 5. The production and distribution of quality virus indexed TC plants of banana and orchids

#### FIVE DECADES OF GLORIOUS RESEARCH\_

The centre is producing good quality tissue culture plants of banana, orchids etc. and distributing throughout Kerala through various Research Centres of the University and directly to farmers.

#### 6. Analysis of soil, plant and water samples.

The centre has excellent facility for the analysis for the estimation of macro, secondary and micronutrients of soil and plant samples, heavy metals of water samples etc. brought by the farmers and other institutions.

## 7. A platform for providing livelihood security and employment to the urban folks.

The centre could provide job opportunity to a good number of neighboring people through its various programmes particularly tissue culture, biofertilizer production etc. which helped for poverty alleviation and enhanced livelihood security.

# Conclusion

This centre was established in the year 1958 exclusively for the salinity research of rice and could accomplish research with respect to the crop improvement aspects through development of high yielding rice varieties, management aspects of saline farming and development of suitable organic management packages for pokkali tract, development of suitable integrated farming models for the Pokkali ecosystem. Further, the centre could diversify its activities and started research on development of orchid hybrids, mass production of tissue culture plants of orchids and banana. Recently, the centre started the commercial production of tissue culture plants of orchids, banana and other ornamentals for distribution throughout Kerala. Further, the centre has started advanced molecular breeding research like Marker Assisted Breeding in rice and introgressed the Saltoi gene into the mega rice variety Jyothi and the introgressed lines are under field evaluation. This work is first of its kind in Kerala and the introgression of abiotic stress tolerant genes / QTL's like Saltol and Sub 1 genes into three mega rice varieties of Kerala viz. Uma, Jyothi and Jaya is progressing. These abiotic stress tolerant QTL introgressed lines are intended to cultivate in all saline prone areas of Kerala including salt intruded areas of Kuttanad and Kole lands. The study of the underlying mechanisms in the different valuable pokkali land races and pyramiding of these different mechanisms into a single rice variety is another major ongoing programme of the station. The search of new saline tolerant genes/QTLs in the existing land races in the germplasm is also another area of research.

The establishment of an analytical laboratory with sophisticated instruments facilitated the centre for the regular analysis of the macro, secondary and micro nutrients in the soil, plant and water samples from different centres of the Kerala Agricultural University, Department of Agriculture and for the farming community as well.

Another major development is the mass production and distribution of virus indexed quality tissue culture plants of banana, orchids and other ornamentals. Large scale production of biofertilizer (*Pseudomonas fluorescence*) is another stepping stone of the centre. It is of great satisfaction to state that this centre could empower women through employment in these various activities particularly tissue culture production and nursery activities which results in poverty alleviation and enhanced livelihood security.



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