PINEAPPLE RESEARCH STATION

Research and Development Report 2011-12

Dr. P.P Joy

KERALA AGRICULTURAL UNIVERSITY

PINEAPPLE RESEARCH STATION

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VAZHAKULAM

Research and Development Report 2011-12

(01.04.2011 to 31.03.2012)

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Executive summary

The Pineapple Research Station, Vazhakulam aims to become the ultimate authority and provider of excellent quality technology, products and services in pineapple and other tropical fruit crops through concerted research and development efforts sustained by best human resource and infrastructure development in line with its Motto 'Quality People & Infrastructure for Quality Technology, Products & Services and Merit alone counts for Quality suitable for the purpose'. The research and development efforts are fine tuned to this effect. The pineapple hybrids produced in the hybridisation programme 'breeding for yield and quality of pineapple' are evaluated. Irradiated suckers of pineapple variety Mauritius were evaluated for better fruit quality and the evaluation of the better types is continued. During last year about 900 hybrids were evaluated for yield and quality parameters. Five hybrid lines produced fruits having weight more than 1.9 kg and TSS more than 18%. Different types found in farmers fields were collected and being evaluated. Various types of passion fruit collected from southern states to identify a passion fruit variety suitable for low altitude areas in Kerala are being evaluated. Passion fruit with accession number 88 possessed maximum rind weight and juice weight followed by accession number 57. Pulp weight was highest for the accession no. 55. Accession number 57 showed highest amount of Ascorbic acid, non reducing sugar and total sugar content which was followed by accession number 88. Qualitative characters analysis showed the passion fruit with accession no. 88 had good taste, colour and size followed by accession number 86 which had best aroma.

Pest and disease problems of 50 farmers were attended to during last year. Work on detection of virus disease in pineapple is continued. Production of tissue culture pineapple is continued and tissue culture Production of passion fruit and banana is augmented. Efforts are being made to standardize protocols for the micropropagation of pineapple, passion fruit and banana. Fresh inoculation of two varieties of pineapple (Mauritius and MD-2) was done in MS+3BA and obtained multiple shoots within a month. Already established cultures were subcultured in MS+4BA+1NAA for multiplication and MS+1IBA+1NAA for rooting at regular time interval. The plants with enough roots were treated with 20g/l pseudomonas for 20 minutes and planted in potting mixture (Cowdung + solarised soil) for hardening. Less rooted plants were treated with 1ml NAA/l for half an hour before planting for further rooting. Nodes of the two varieties (purple and yellow) of passion fruit gave maximum response in 2BA. Fresh inoculation of five varieties of banana (Red banana, Nendran, Robesta, Poovan and Njalipoovan) was done in MS+3BA. Multiplication of the buds (MS+3IBA+1.5BA) was observed within 120 days. Rooting was maximum in 1/2MS+2mg/l NAA+0.5g/l activated charcoal.

An externally aided project on 'Evaluation of passion fruit types for commercial cultivation in Kerala' at a total cost of Rs.12.55 lakh for 3 years is sanctioned by Kerala State Council for Science, Technology and Environment to harness the full potentials of the growing situation giving maximum benefit to the growers in terms of more employment, higher incomes and better standard of living enabling better food, health and employment security. Memorandum of understanding was written between KAU and Kerala State Council for Science, Technology and Environment. The project is being undertaken. Another project proposal to establish a fruit processing laboratory at PRS, Vazhakulam for the efficient conversion of leftover fruits to value added products like squash, jam, syrup, etc at a total cost of Rs.19.90 lakh was submitted for approval under RKVY 2011-12.

A development plan of research station was submitted to University, Agricultural Minister, Revenue Minister, Collector of Ernakulam, Sri. Joseph Vazhakkan, MLA, Muvattupuzha, District Panchayath President and Block Panchayath President. Earnest efforts are taken to obtain free revenue land as research farm for the station. Pineapple Research Station, Vazhakulam prepared its Vision 2030 wherein it visualizes to be Tropical Fruit Crops Research Station (TFCRS) in the near future. The advanced research centre of excellence dreams to be the ultimate authority and provider of excellent quality technology, products and services in fruit crops through concerted research and development efforts sustained by best human resources and infrastructure development.

The management problems faced by pineapple farmers are regularly attended by visiting fields, in person, seminars, through telephones, emails etc. Extension activities are mainly done in association with the Pineapple Farmers' Association. The websites of the station www.kau.edu/prsvkm and prsvkm.tripod.com were updated with more relevant and useful information for the public. As per the instruction of the director of research during his inspection of the station on 12-01-2012 leaflets on Pineapple Research Station both in English and Malayalam were prepared. Leaflets on pineapple and passion fruit in Malayalam were prepared and got printed for distribution to the public.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

RESEARCH AND DEVELOPMENT REPORT OF PINEAPPLE RESEARCH STATION, VAZHAKULAM FOR 2011-2012

A. STATION AT A GLANCE

The Pineapple Research Station at Vazhakulam was established on 2nd January 1995 to give research and development support to pineapple farmers. Since then, this research centre of the Kerala Agricultural University has been steadily growing and serving as a subvention to the pineapple growers of the state and the country as well. The centre had a humble beginning as "Pineapple Research Station & Pest and disease Surveillance Unit" under Kerala Horticulture Development Program (KHDP). For the construction of the office-cum-laboratory building of the station, 15 cents of land was transferred from the Revenue Department to Kerala Agricultural University on 24.6.1996. It was delinked from KHDP and became a constituent research centre of Kerala Agricultural University under central zone on 1.7.1997. The present building was occupied on 27.6.1998.

Our Mission

To be the ultimate authority and provider of excellent quality technology, products and services in the pineapple and other tropical fruits sector through concerted research and development efforts sustained by best human resource and infrastructure development

Mandate

- Give research and development support to the pineapple cultivators
- Provide quality technology, products and services to the pineapple sector
- Undertake basic and applied research in pineapple and other fruit crops of Kerala

Achievements

The centre undertakes basic and applied research and development activities in pineapple and other fruit crops of Kerala. The research and development projects are mainly in Participatory technology development (PTD) mode and funded by various agencies as KAU, State and central governments, ICAR, SHM, NHM, KSCSTE, etc. The station has taken up research in pineapple on various aspects like intercropping in rubber and coconut, plant spacing and density, organic and chemical fertilizer requirement etc, besides experiments on development of new varieties. The centre has developed scientific technology for the commercial cultivation of Kew and Mauritius varieties of pineapple, including pure cropping, intercropping in rubber and coconut plantations and in paddy lands. Technology is also developed for organic production. Based on continuous surveillance and laboratory studies the station has identified the presence of pineapple mealy bug wilt associated (PMWA) virus in Vazhakulam area. Based on all the findings, this station has formulated the Package of Practices Recommendations for the popular varieties Mauritius and Kew and included in the KAU POP and all the technology developed are being transferred to the pineapple growers extensively. Tissue culture protocols for various varieties of pineapple, passion fruit and banana are

available. Vazhakulam pineapple has been registered in the Geographical Indication Registry to boost the export of pineapple. The station is pursuing its User Registration. Participatory technology process and product development in association with sister institutions, Nadukkara Agro Processing Co. Ltd. and Pineapple Farmers' Association for the stake holders is a steady and continuing process at the centre. The station has already produced and sold more than 60,000 Tissue Culture pineapple plants and 25,000 passion fruit seedlings. Large scale tissue culture production of banana has been started. Pineapple Research Station launched its own website (www.kau.edu/prsvkm) as a subsite under the Kerala Agricultural University main site in June 2010. The websites of the station www.kau.edu/prsvkm and prsvkm.tripod.com were updated with more relevant and useful information for the public facilitating free download of the publications of the centre.

Facilities

Laboratory: Plant biotechnology, phytochemistry and microbiology labs equipped with Gel documentation unit, ELISA Reader & washer, PCR, UV visible spectrophotometer, UV-Transilluminator, Flame photometer, Centrifuge, Microscopes, Electrophoresis unit, Shakers, ovens, Precision Weighing balances, Deep freezer, BOD incubator, Laminar Air Flow chambers, still, etc

***Farm*:** 1.2 hectares

***Library*:** Specialised books and periodicals relevant to the sector

***Sales Centre*:** For the public sale of Tissue Culture Plants, Seedlings, Rooted cuttings, Publications, etc

Research

The centre undertakes basic and applied research and development activities in pineapple, passion fruit, banana and other fruit crops of Kerala. The research and development projects are mainly in Participatory technology development (PTD) mode and funded by various agencies as KAU, State and central governments, ICAR, SHM, NHM, etc.

Participatory Technology Development

The centre has developed scientific technology for the commercial cultivation of Kew and Mauritius varieties of pineapple, including pure cropping, intercropping in rubber and coconut plantations and in paddy lands. Technology is developed for organic production. Tissue culture protocols for various varieties of pineapple are available. GI indication of Vazhakulam Pineapple is registered. Participatory Technology Process and product development in association with sister institutions, Nadukkara Agro Processing Co.Ltd. and Pineapple Farmers' Association for the stake holders is a steady and continuing process at the centre.

Seed & Nursery

The station undertakes large scale production of Tissue Culture Plants of different varieties of Pineapple, Passion fruit and Banana and Seedlings and Rooted cuttings of Passion fruit. They are available for sale at the centre. Booking for the planting materials can be made with advance payment as Demand Draft in favour of Associate Professor & Head, PRS, Vazhakulam payable at State Bank of India, Vazhakulam-686670, Muvattupuzha, Ernakulam, Kerala (Code No: 7844). Priority is always given to firm orders with advance payment and delivery will be on first-come-first-serve basis.

Extension

Technology transfer is effectively carried out through personal discussions, field visits, phones, emails, website, posts, radios, TVs, news papers, periodicals, publications, pineapple fests, seminars, trainings, etc. Publications such as leaflets, palmlets, books, CDs, DVDs, etc covering various aspects of cultivation and utilization of the mandatory crops of the station are also being undertaken.

Products

- Tissue Culture Plants of pineapple, passion fruit and banana
- Seedlings of passion fruit
- Rooted cuttings of passion fruit
- Publications

Services

- Agriclinic & advisory
- Training
- Consultancy
- Quality testing
- Project work of U.G. & P.G. students of other Universities
- Large scale Tissue Culture production

Staff

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Sri. Justin T. Jose, Senior Grade Assistant, +919744469876
Daily wage contract skilled assistants and labourers

Looking ahead

Earnest efforts are also being taken to acquire free government land nearby as a permanent farm for raising various fruit plants, conserving germplasm and conducting field research, besides establishing adequate infrastructure for further development and diversification, renaming the station as Tropical Fruit Crops Research Station (TFCRS). It is also

proposed to establish a fruit processing laboratory with FPO registration at the centre for the efficient conversion of leftover fruits to value added products like squash, jam, syrup, etc.

Besides pineapple, since Vazhakulam and neighboring areas are well-known for other fruit crops like banana, mango, jack, papaya, passion fruit, rambutan, mangosteen, etc, and there is no research station in the district catering to the needs of these farmers, Pineapple Research Station, Vazhakulam visualizes to be Tropical Fruit Crops Research Station (TFCRS) in the near future. This advanced research centre of excellence dreams to be the ultimate authority and provider of excellent quality technology, products and services in tropical fruit crops through concerted research and development efforts sustained by best human resource and infrastructure development in line with Our Motto 'Quality People & Infrastructure for Quality Technology, Products & Services and Merit alone counts for Quality suitable for the purpose'.

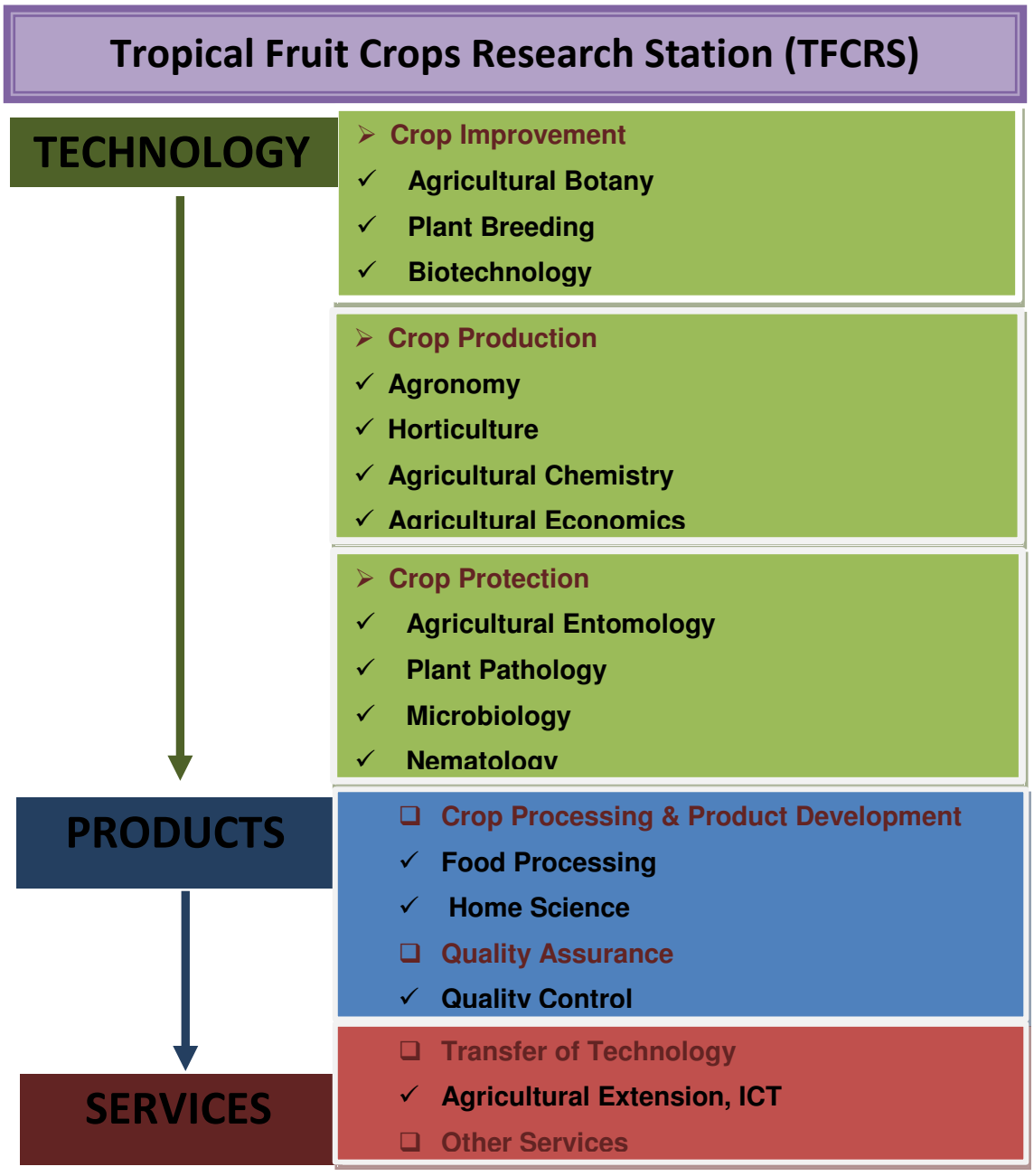


Fig. 1. Prospective Structural Hierarchy of the Tropical Fruit Crops Research Station

B. ONGOING PROJECTS

The following Plan projects are ongoing during 2011-12.

321-31-3370: Research on pineapple

321-31-3500: Research in passion fruit

321-31-4449: Breeding for yield and quality of pineapple

321-31-8841: Selection of high yielding superior quality pineapple variety for central zone of Kerala in PTD mode

A development plan was submitted to the university depicting the station at a glance, narrating the urgent felt-needs of the station and proposing a metamorphosis into Tropical Fruit Crops Research Station (TFCRS) in line with Our Motto 'Quality People & Infrastructure for Quality Technology, Products & Services; Merit alone counts for Quality suitable for the purpose and one has know-how only when it is proven in real life'.

C. DETAILED RESEARCH REPORT

1. RESEARCH ON PINEAPPLE

Pineapple research station is actively involved in genuine efforts for standardization of the tissue culture protocols meant for the mass production of three varieties of pineapple namely MD-2, Mauritius and Amritha.

Plant Tissue Culture, is a well advanced micro propagation technique for the mass production of any plant part, in a specially defined growth medium under aseptic laboratory conditions. Tissue culture depends on the plasticity and totipotency of the plants. Plasticity triggers one type of tissue or organ to be initiated from another type under proper external stimuli, where as totipotency maintains the total expression of the complete genetic profile. Tissue Culture technique involves four major stages, namely, *Initiation (Fresh inoculation), Mass Production (Multiplication), Rooting and Hardening (Plant out).*

1.1 Micropropagation of MD-2, Mauritius and Amritha

Fresh inoculation of Amritha variety was not done in the station, as we have obtained the cultures from RARS, Pattambi, Kerala Agricultural University.

Stage 1. Fresh inoculation of MD-2 and Mauritius sucker explants

Explant Source: Field of Pineapple Research Station, Vazhakulam

Objective

To freshly inoculate sucker explants of MD-2 and Mauritius for micropropogation

Technical programme

For surface sterilization unwanted leaves, roots and soil exposed parts of sucker were chopped off. The meristematic shoot apices were exposed to running water for 30 minutes. Explants were further cleaned using Teepol wash in distilled water for 30 minutes. After several

rounds of wash using soap solution, explants were trimmed and were dipped in Bavistin (0.1%) + Indofil (0.1%) + SAAF (0.05%) for 30 minutes. Subsequent to distilled water wash, explants were again trimmed and treated with 2ml/l Gentamycin for 1 hour. Prior to inoculation, explants were trimmed and surface sterilized in laminar air flow cabinet with 70% ethanol for 2 minutes. Explants were given single sterile distilled water wash. Later on they were treated with 0.1% (w/v) aqueous mercuric chloride for 5 minutes followed by 3 times sterile distilled water wash, each for a duration of 5 minutes. The exposed portions of the explants were cut off and were dipped in antibiotic Gentamycin solution.

The beaker was kept inside laminar air flow cabinet and resized explants were taken out using sterile forceps. The exposed portions of the explants were smoothly removed with sterile forceps and scalpel. The sterile MD-2 explants were inoculated into the medium in test tubes. Tubes were carefully tightened and properly labeled before transferring to incubation room maintained at 25-27^{o}C.

Results

The freshly inoculated MD-2 cultures were observed for a cycle of 21 days. After 7days they appeared in slight green colour. Within 14 days they turned green. By 21 days the bud number increased from 1 to 3. Callus formation was absent and hence, for multiplication another medium must be provided.

The freshly inoculated Mauritius cultures were also observed for a cycle of 21 days. After the 7th day they appeared green. Within 14 days new buds sprouted and bud number increased from 1 to 3 in 21 days. For Mauritius, since the same medium supported callus formation, fresh medium was used for multiplication.

Table 1. Intensity of bud production 21 days after inoculation of MD-2 and Mauritius in different media

Pineapple Variety		Media	No. of buds after 21 days	Response
MD-2	(a)	MS+4 mg/l BA+1mg/l NAA	2	+ +
	(b)	MS+3mg/l BA	3	+ + +
Mauritius	(c)	MS + 5 mg/L BA	2	+ +
	(d)	MS+1.5mg/l BA+0.01mg/l NAA	1	-
	(e)	MS+0.5mg/l NAA+2 mg/l kinetin	1.5	+
	(f)	MS+4 mg/l BA+ 1mg/l NAA	3	+ + +
	(g)	MS+4.44µMBA	0	-

No change (-), Minimum (+), Medium (++), Maximum (+++)

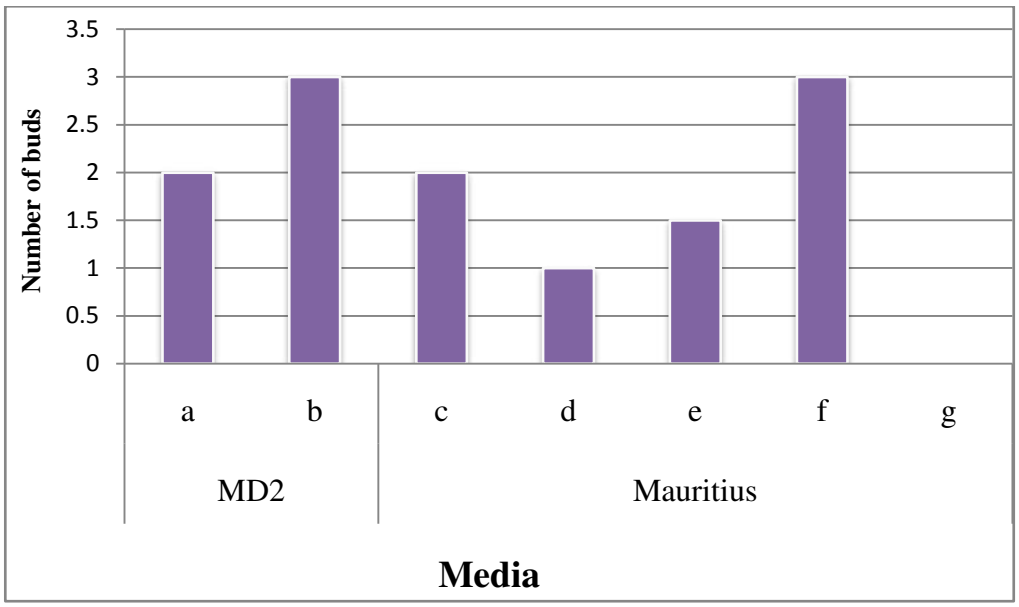


Fig. 2. Intensity of bud production 21 days after inoculation of MD-2 and Mauritius in different media

Stage 2. Multiplication

Objective

To identify and standardize suitable multiplication medium for the varieties MD-2, Mauritius and Amritha

Technical Programme

For the multiplication of MD-2 , MS+3mg/lBA medium was not effective and so MD-2 cultures were subcultured to MS+4mg/lBA+1mg/lNAA medium for increased callus and bud formation. Enough cultures were obtained after 6th subculture and for shoot formation they were subcultured in MS+3mg/lBA+0.5 mg/lNAA medium.

Since MS+3mg/lBA medium was found ineffective for active multiplication of Mauritius, they were subcultured to MS+4mg/lBA+1mg/lNAA multiplication medium. Mauritius showed active bud development in MS+4mg/lBA+1mg/lNAA medium. For effective callus formation MD-2 was again sub-cultured in the same medium up to 7th subculture.

Amritha callus were subcultured in four different media to identify effective multiplication medium. At the plant out stage they were subcultured to Full MS (FMS) medium for shoot formation.

Results

1st and 2nd subculture stages of MD-2 showed increased bud numbers in 14 days. 3rd to 6th subcultures displayed increased callus formation. MD-2 callus subcultured in MS+3mg/l BA+0.5 mg/l NAA medium was observed to have slight green shoots within 14 days. Slight green was changed to green after 45 days.

Mauritius buds during 1st and 2nd subculture showed colour change from green to slight green, as a start to callus formation after 7days. By the end of 14 days white creamy callus formation was observed. Continued growth in the same medium for 45 days led to green shoots formation.

Amritha callus subculture in MS + 2mg/l BA + 1mg/l NAA showed increased callus formation after 21 days. All other media trials gave medium and minimum responses. Extended growth in the same medium for 45 days led to development of green shoots.

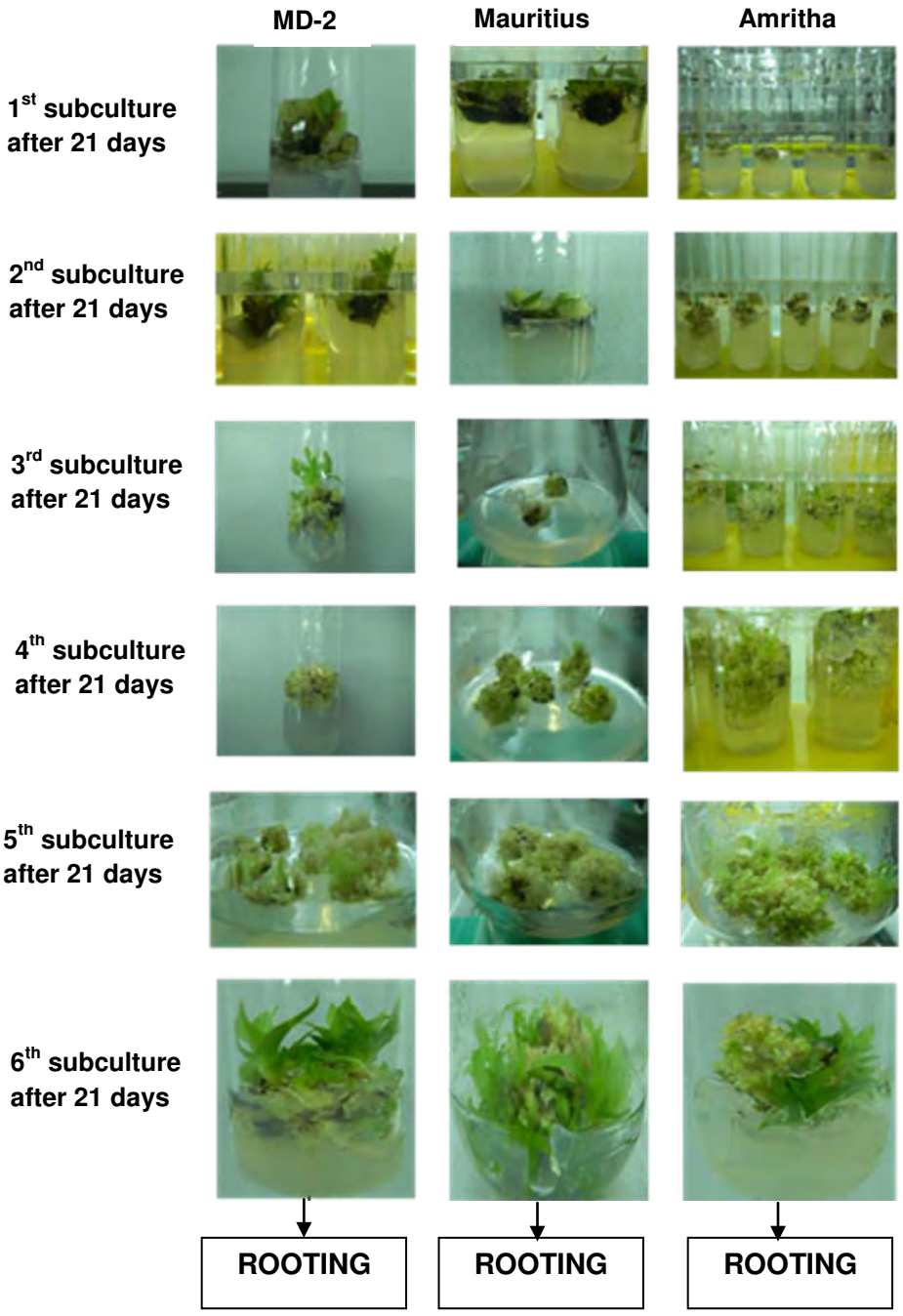


Fig. 3. Sequential subculturing and multiplication of MD-2, Mauritius and Amritha pineapple

Table 2. Response of pineapple varieties to different multiplication media

Pineapple Variety	Media	After 21 days	Response
MD-2	(a) MS + 4mg/l BA + 1mg/l AA	Increased callus formation	+ + +
	(b) MS + 3mg/l BA + 0.5 mg/l NAA	Green shoots obtained	+ + +
Mauritius	(c) MS + 4mg/l BA + 1mg/l NAA	Increased callus formation	+ + +
Amritha	(d) MS + 4mg/l BA + 1mg/l NAA	Medium callus formation	+ +
	(e) MS + 2mg/l BA + 1mg/l NAA	Increased callus formation	+ + +
	(f) MS + 1mg/l BA +2mg/l NAA	Minimum callus formation	+
	(g) MS + 3mg/l BA + 0.5 mg/l NAA	No change	-

No change (-), Minimum (+), Medium (++), Maximum (+++)

Stage 3. Rooting

Objective

To identify and standardize suitable rooting medium for MD-2, Mauritius and Amritha shoot tips.

Technical Programme

In order to subculture MD-2 in rooting medium, green shoots were separated using sterile forceps and inoculated to various media for shoot elongation and rooting. Media included FMS, Half MS (HMS), HMS+1g/l activated charcoal, HMS+2mg/l IBA, HMS+1.5mg/l IBA, HMS+2mg/l NAA, HMS+1mg/l IBA+1mg/l NAA, HMS+1.5mg/l IBA+1mg/l NAA, HMS+1.5mg/l IBA+2mg/l NAA, HMS+1.5mg/l IBA+1.5mg/l NAA, HMS+2mg/l IBA+1mg/l NAA, HMS+2mg/l IBA+2mg/l NAA and HMS+2mg/l IBA+1.5mg/l NAA.

Green shoots from Mauritius subculture was separated using sterile forceps and inoculated to FMS and HMS media for shoot elongation and rooting. Hormone-less medium was tried to get enough rooting.

Amritha shoots were obtained from the subculture in MS + 2mg/l BA + 1mg/l NAA medium. They were separated using sterile forceps and were inoculated to HMS medium containing rooting hormone. Hormone-less HMS and FMS media were also tried for rooting experiments.

Results

MD-2 shoot formation and elongation was very good in HMS + (1mg/l IBA+1mg/l NAA) medium. HMS+ (1.5mg/l IBA +1.5mg/l NAA) medium exclusively supported rooting, whereas medium number of roots were observed in HMS+ (2mg/l IBA +1.5mg/l NAA) medium. Shoot initiation observed in HMS+ (1.5mg/l IBA) medium but was lesser than the results from HMS+ (1.5mg/l IBA +1.5mg/l NAA) medium. HMS+(1g/l activated charcoal), HMS+ (2mg/l IBA), HMS+(1.5mg/l IBA +1mg/l NAA), HMS+(2mg/l IBA +1mg/l NAA), HMS+(1.5mg/l IBA +2mg/l NAA), HMS+(2mg/l IBA +2mg/l NAA and HMS+(2mg/l NAA) media showed minimum responses for rooting.

Mauritius shoots showed more rooting in half MS medium than in full MS medium. Rooting rate and number of roots increased by the 45thday.

Amritha shoots showed highest number of roots in HMS+2mg/lIBA+1.5mg/l NAA medium after duration of 45 days. Response of Amritha variety to HMS+1mg/l IBA was medium. In FMS and HMS media they exhibited minimal rooting.

Table 3. Periodical root production in MD-2 tissue culture plants in different rooting media

Medium	Number of roots in nth day				Response
	7	14	21	45	
(a) FMS	0.00	0.00	0.00	0.00	-
(b) HMS	0.00	0.00	0.00	1.00	+
(c) HMS+1g/l activated charcoal	0.00	0.00	1.00	1.00	+
(d) HMS+2mg/l IBA	0.00	0.00	0.00	0.50	+
(e) HMS+1.5mg/l IBA	0.00	0.00	0.25	0.75	+
(f) HMS+2mg/l NAA	0.00	0.25	0.25	0.50	+
(g) HMS+1mg/l IBA+1mg/l NAA	0.00	1.00	2.00	5.00	+++
(h) HMS+1.5mg/l IBA+1mg/l NAA	0.00	1.00	2.75	3.50	+
(i) HMS+1.5mg/l IBA+2mg/l NAA	0.00	0.50	1.75	2.75	+
(j) HMS+1.5mg/l IBA+1.5mg/l NAA	0.50	1.50	2.50	5.50	+++
(k) HMS+2mg/l IBA+1mg/l NAA	0.00	0.75	2.00	3.00	+
(l) HMS+2mg/l IBA+2mg/l NAA	0.00	1.00	1.25	2.50	+
(m) HMS+2mg/l IBA+1.5mg/l NAA	0.00	1.00	1.50	4.00	++

No change (-), Minimum (+), Medium (++), Maximum (+++)

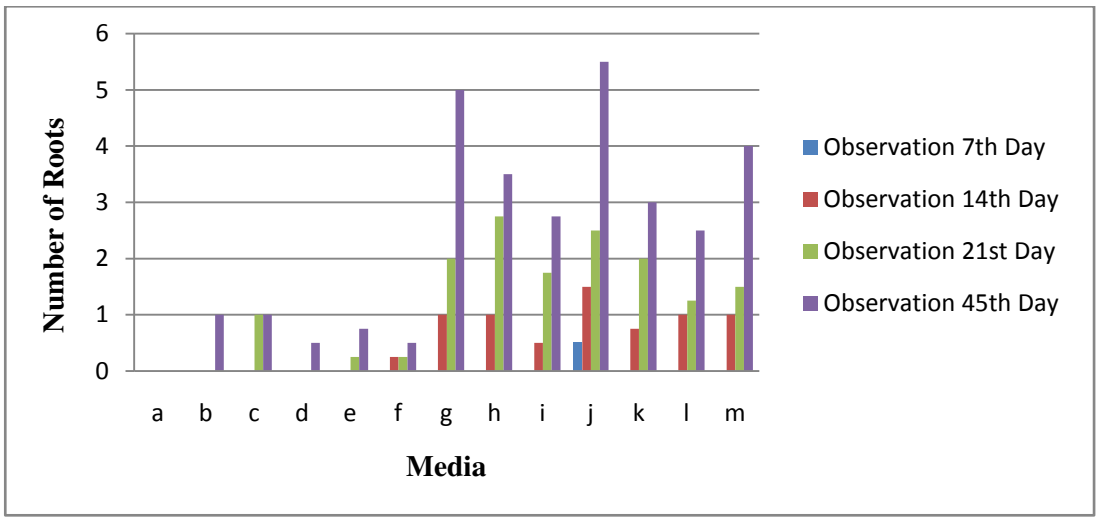


Fig. 4. Periodical root production in MD-2 tissue culture plants in different rooting media

Table 4. Periodical root production in Mauritius tissue culture plants in different rooting media

Media	Number of roots in n^{th} day				Response
	7	14	21	45	
(a) FMS	0	0	0	0	-
(b) HMS	0	0	1	2	+ +

No change (-), Minimum (+), Medium (++), Maximum (+++)

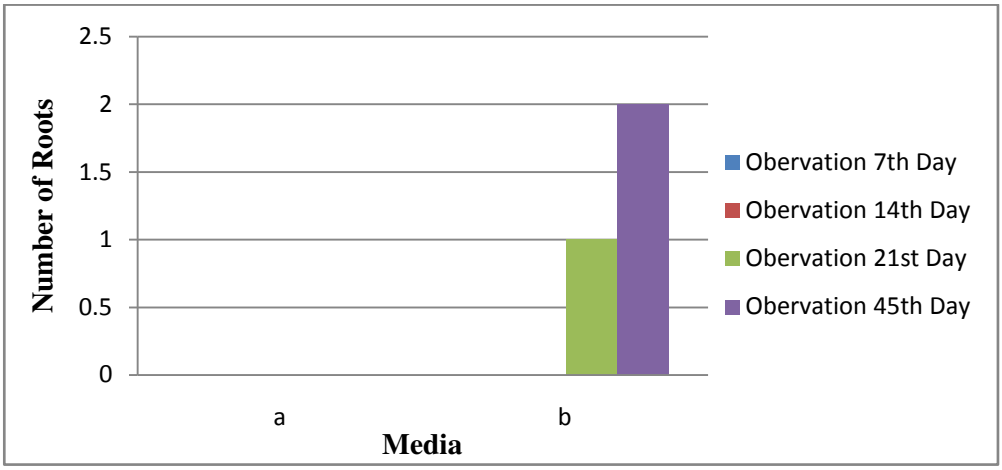


Fig. 5. Periodical root production in Mauritius tissue culture plants in different rooting media

Table 5. Response of Amritha pineapple to Different Rooting Media

Media	Number of roots in nth day				Response
	7	14	21	45	
(a) FMS	0	0	0	0	-
(b) HMS	0	0	0	1	+
(c) HMS+1mg/l IBA	0	0	1	1.5	+ +
(d) HMS+2mg/lIBA+1.5mg/l NAA	0	1	1.5	4	+ + +

No change (-), Minimum (+), Medium (++), Maximum (+++)

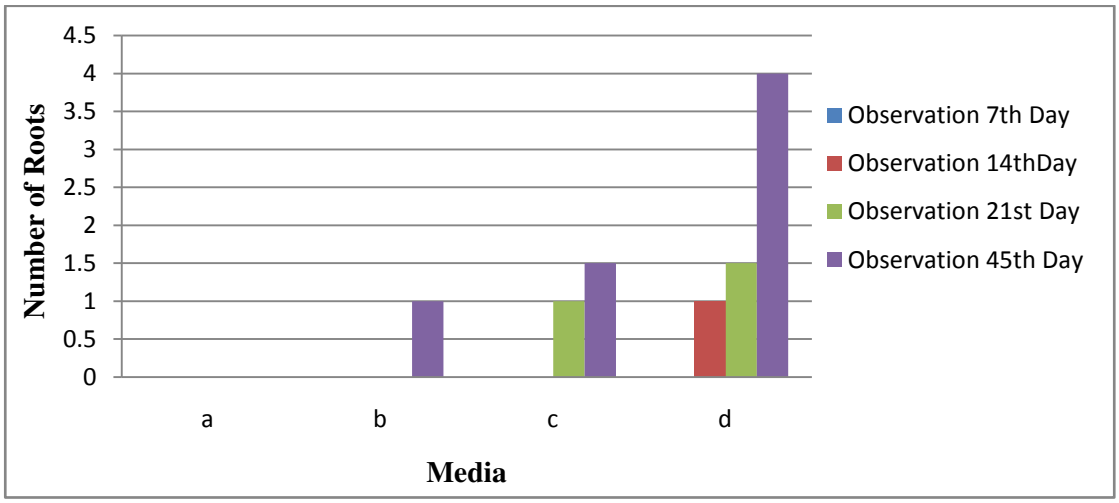


Fig. 6. Periodical root production of Amritha pineapple in different media

Stage 4. Hardening of Pineapple

Fully rooted plants *in vitro* were selected for plant out. Plants were first grown in mist chamber for acclimatizing with the climate outside the lab. After 2 to 3 week's time they were moved to green house to get adjusted with field conditions. Healthy plants were treated with pseudomonas 2g/l for 30 minutes and planted in potting mixture. Contaminated plants were treated with 2g/l SAAF for 30 minutes and planted.

Potting mixture was made by mixing 100 kg solarised Soil+10 kg Cowdung+1 kg Neem Cake. The mixture was thoroughly mixed and irrigated well. Potting mixture was kept covered for 4-5 days and Trichoderma (1-2 kg) was added. It was again mixed and irrigated well. Mixture was kept covered. For one week it was irrigated and mixed at 2 days interval. This mixture was further used for planting.

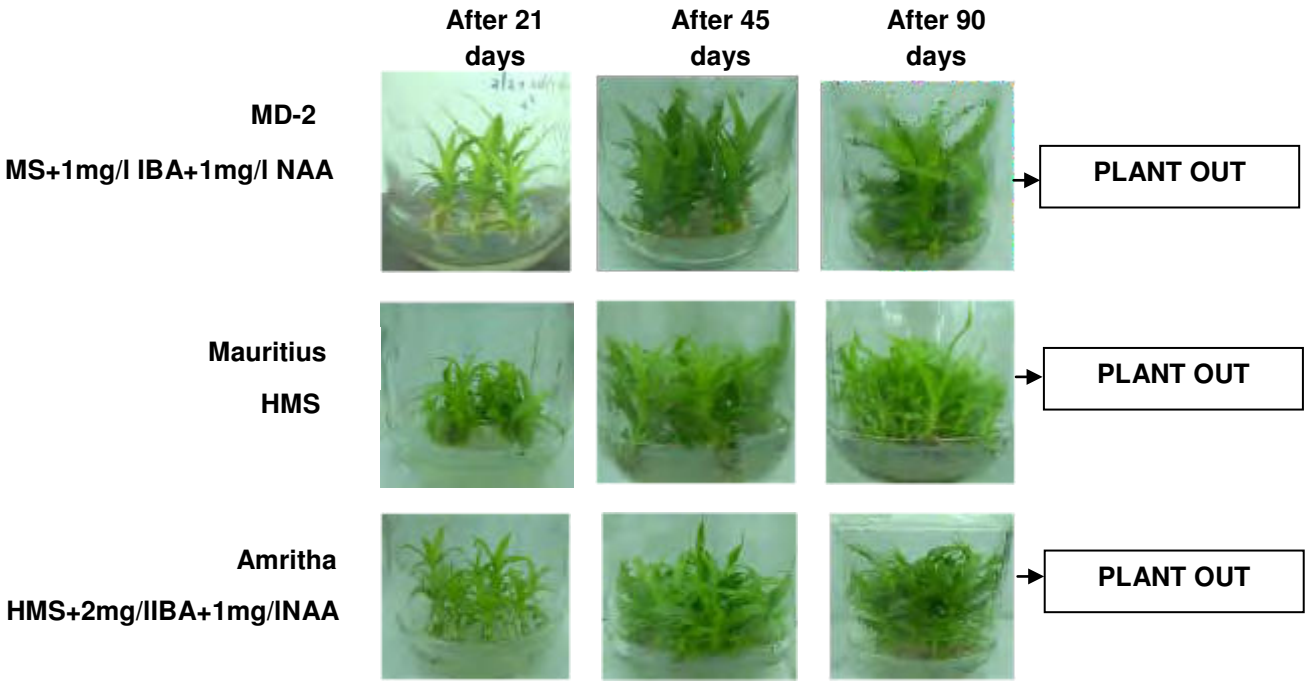


Fig. 7. Rooting of MD-2, Mauritius and Amritha

Fig. 8. Plant Out of MD-2, Mauritius and Amritha after 3 months

1.2 Breeding for Yield and Quality of Pineapple

Objective

To develop pineapple varieties suitable for processing and table purpose through hybridization

Technical programme

The project was initiated in 2002. The traditional pineapple varieties of Kerala Kew and Mauritius were hybridized and F1 hybrids were planted in the field and selections were made based on favorable yield and qualitative characteristics. The suckers of superior types were subsequently planted in the field and the evaluation is being carried out continuously. Observations on fruit weight with and without crown, crown weight and TSS were being taken and the data were utilized for the selection of superior types.

Result

The following observations were taken and the data corresponding to superior varieties are furnished below. Five hybrid lines produced fruits having weight more than 1.4 kg and TSS more than 18%. The evaluation is being continued. The planted lines are over three years now and need to be replanted.

Table 6. Pineapple Hybrid Line Performance in 2011-12

Plant no.	Fruit + Crown (g)	Crown wt. (g)	Fruit wt. (g)	TSS (%)
11204(4-59)	1813.50	192.00	1621.50	22.0
2882(4-58)	1842.00	356.50	1485.50	18.0
802(4-24)	1635.00	205.50	1429.50	18.6
2731(4-13)	1635.00	205.50	1429.50	18.6
1261(4-40)	1535.50	123.00	1412.50	19.6

Evaluation of Shortlisted pineapple hybrid lines

Technical programme

After observing the available data on the progenies recorded in the basic records and field books, The Associate Director of Research, RARS, Pattambi during his inspection on 22/07/11 has directed to short list the unwieldy number of accessions into a manageable group of 100- 200 numbers for the next stage of evaluation. Subsequently the best promising 10-12 numbers can be agronomically evaluated in RBD to arrive at one or two good varieties in pineapple which can be recommended for release.

Accordingly, the data for the last three years ie, 2008-09, 2009-10 and 2010-11 were analyzed and the top 50 performers were selected separately for each year based on fruit weight and brix value. All the accessions for which the detailed quality analysis report was available were also included in the list.

Entire accessions which satisfied the criteria were pooled and sorted. Overlapping accessions were checked in the experimental plot for availability of suckers, which can be used for replanting. Finally 186 superior plants were selected for replanting and further evaluation. A maximum number of five suckers (A, B, C, D and E) of the available ones were planted in plot 1. The crop was managed as per the KAU package of practices recommendations.

Experimental programme followed for the entire replanted accession numbers can be broadly classified as analysis of yield characters, phytochemical characters and qualitative characters. Yield character studies included detection of number of fruits under each accession numbers, calculation of fruit weight, rind weight, pulp weight, seed weight and juice weight. Phytochemical analysis quantified the TSS, pH, acidity, ascorbic acid, reducing sugars, non-reducing sugars and total sugar. Taste, colour, size and aroma of the fruits were qualitatively scored in 0-9 scale.

Results

The analysis pertains to the initial harvest of fruits which did not get optimum period for growth and development, as the planting was taken up recently only.

Yield character analysis of pineapple accessions identified the pineapple with accession no. 2011 which had the highest weight with crown, juice weight and pulp weight. Fruit weight was highest for the accession number 5004. Accession number 549 had the lowest peel weight. Accession number 833 had the lowest core weight.

Analysis of the phytochemical characters identified plant number 549 had highest TSS followed by plant number 5004. pH value was lowest for plant number 833. Plant number 2011 showed the highest percentage of ascorbic acid, reducing and total sugar.

The data on pineapple accessions in the year 2011-12 are tabulated as below.

Table 7. Yield Characters of Pineapple Accessions

Plant No.	Fruit+ Crown(g)	Crown Weight(g)	Fruit wt(g)	Peel wt(g)	Core wt(g)	Juice wt(g)	Pulp wt(g)
2011	726.00	226.00	500.00	84.00	64.00	348.50	229.50
549	288.50	62.50	226.00	47.00	54.00	132.00	55.00
5004	682.00	70.00	612.00	126.00	57.00	309.00	190.00
833	391.17	104.83	286.87	64.37	45.00	104.37	149.28

Table 8. Phytochemical Characters of Pineapple Accessions

Plant No.	TSS (%)	pH	Acidity (%)	Ascorbic acid (mg/100g)	Reducing sugar (%)	Non red. Sugar (%)	Total sugar (%)
2011	15.00	4.03	0.60	59.09	3.78	15.07	18.85
549	28.00	3.82	0.78	52.26	3.68	14.73	18.41
5004	22.60	3.94	0.70	41.17	3.33	17.16	20.49
833	20.05	3.70	0.65	37.82	2.61	12.44	15.06

Table 9. Qualitative Characters of Pineapple Accessions (0-9 scale)

Plant No.	Taste	Colour	Size	Smell
549	8.50	5.00	1.00	5.50
833	4.75	3.00	3.63	3.38
2011	2.50	4.00	3.50	3.00
5004	6.00	5.00	3.00	4.00

1.3 Selection of High Yielding Superior Quality Pineapple Variety for Central Zone of Kerala in PTD Mode

Objective

To select a high yielding superior quality pineapple variety for central zone of Kerala

Technical programme

The participatory technology development (PTD) research programme encompasses a number of modules like survey, collection, screening, evaluation with farmers and participatory approach involving Pineapple Farmers' Association in Kerala. Field experiments will be undertaken to achieve the various objectives of the project.

Survey, collection and conservation of elite pineapple types

The different elite pineapple types available with Pineapple Farmers' Association, farmers and institutions in the state will be collected, established and conserved in the research center.

Characterization of elite pineapple types

The different elite types available with Pineapple Farmers' Association, farmers and institutions in the state will be established, multiplied and used for characterization of plant types. The types will be characterized morphologically and biochemically.

Identification of suitable pineapple types for cultivation

The collection of elite pineapple types available at Pineapple Research Station and those collected from Pineapple Farmers' Association, farmers and institutions in the state, established at the center will be evaluated for their growth, yield and quality characteristics. A suitable yield index will be developed with the participation of Pineapple Farmers' Association and different types will be ranked according to the yield index. The top three promising one will be evaluated in detail for their quality and acceptance by Pineapple Farmers' Association, farmers and institutions.

Results

Observations were taken every four months and growth parameters were recorded. After four months of planting Mauritius showed highest plant height, canopy spread, no. of leaves and leaf width. Normal suckers were used as planting material for Mauritius and the initial growth pace may be because of that. For all other accessions tissue culture plants were used for planting, which is characterized by slow initial growth compared to normal suckers. Among the tissue culture plants H-5 and MD-2 recorded higher growth parameters.


Fig. 9. Mauritius, Kew and MD-2 in plot 5 (Varietal experiment)

Fig. 10. Field staffs taking observations on growth parameters

Observation taken after 8 months also witnessed Mauritius significantly superior to all other accessions with the highest values for all growth parameters. The best performance of Mauritius can due to the fact that normal suckers were used as planting materials whereas for others tissue culture plants were used for planting. Mauritius was followed by MD-2, H-4 and H-5. Growth parameters were poorest for H-2 followed by H-1 and H-3.

Growth parameters observed after 12 months displayed the accessions of Mauritius and MD-2 being superior in plant height, canopy spread, leaf length and leaf width. Mauritius was significantly superior to all accessions in the total number of leaves. The accession H-2 recorded poorest growth followed by H-1 and H-3.

After 16 months the accessions of Mauritius, MD-2 and Kew faired superior in plant height, canopy spread and leaf length. Number of leaves was highest in H-4, followed by MD-2 and Kew. Leaf width was highest in MD-2 followed by T-3 and Kew. The accession H-2 recorded poorest growth.

By 20 months, Kew recorded best growth performance. H-1 and H-2 showed the least values for all growth parameters and the number of leaves was highest in H-4 followed by MD-2 which was on par.

Table 10. Growth parameters of pineapple accessions 4 months after planting

No	Accessions	Plant Height (cm)	Canopy Spread (cm)	No. of Leaves	Leaf length (cm)	Leaf width (cm)
1	Mauritius	69.80	94.80	36.13	64.53	4.87
2	Kew	27.37	57.40	19.80	32.93	4.11
3	MD-2	36.00	63.27	22.47	40.73	4.35
4	MTS	25.00	51.80	17.13	30.80	2.89
5	T-3	24.93	57.80	17.00	32.93	4.17
6	H-1	16.67	46.87	15.93	25.87	2.81
7	H-2	11.27	23.47	10.93	12.67	1.77
8	H-3	21.60	48.80	18.33	26.67	2.38
9	H-4	25.67	54.33	27.40	28.53	3.50
10	H-5	36.07	68.67	19.73	41.53	2.37
11	Amritha	25.60	49.40	14.93	30.47	2.07
	GM	29.14	56.05	19.98	33.33	3.21
	SEM	2.214	2.806	1.015	1.986	0.275
	CD (0.05)	6.267	8.277	2.995	5.859	0.813
	CV%	12.626	8.670	8.801	10.320	14.870

Table 11. Growth parameters of pineapple accessions 8 months after planting

No	Accessions	Plant Height (cm)	Canopy Spread (cm)	No. of Leaves	Leaf length (cm)	Leaf width (cm)
1	Mauritius	82.93	113.93	49.07	71.80	6.00
2	Kew	39.40	62.87	26.20	39.47	3.63
3	MD-2	48.67	80.60	30.20	40.67	5.07
4	MTS	37.60	56.60	26.07	36.73	4.00
5	T-3	39.33	64.33	23.57	36.83	3.70
6	H-1	20.53	41.87	16.73	21.47	2.53
7	H-2	10.40	23.73	10.33	15.60	1.27
8	H-3	27.80	48.87	20.47	29.60	2.60
9	H-4	37.33	72.20	30.73	36.80	4.10
10	H-5	40.20	68.20	18.07	39.87	2.90
11	Amritha	34.53	59.27	16.17	35.60	2.67
	GM	38.07	62.95	24.33	36.77	3.50
	SEM	4.103	5.591	1.577	3.887	0.282
	CD (0.05)	12.103	16.492	4.652	10.640	0.832
	CV%	18.668	15.382	11.226	16.992	13.972

Table 12. Growth parameters of pineapple accessions 12 months after planting

No	Accessions	Plant height (cm)	Canopy Spread (cm)	No. of Leaves	Leaf Length (cm)	Leaf Width (cm)
1	Mauritius	86.80	107.47	73.33	63.80	5.83
2	Kew	68.40	92.40	28.60	60.13	4.50
3	MD-2	87.07	99.60	31.73	68.67	5.20
4	MTS	66.40	80.33	30.00	58.73	4.20
5	T-3	63.93	92.13	25.20	56.79	4.53
6	H-1	21.00	36.47	17.40	19.80	2.43
7	H-2	15.50	26.27	11.80	16.13	1.63
8	H-3	39.53	61.47	19.67	39.60	3.63
9	H-4	50.37	84.07	31.73	45.20	3.77
10	H-5	75.53	82.47	22.67	55.27	3.60
11	Amritha	45.81	61.60	20.73	41.47	3.07
	GM	56.45	74.93	28.44	47.44	3.81
	SEM	6.487	7.423	2.613	4.842	0.272
	CD (0.05)	19.138	21.897	7.707	14.285	0.822
	CV%	19.907	17.97	15.909	17.559	12.356

Table 13. Growth parameters of pineapple accessions 16 months after planting

No	Accessions	Plant Height (cm)	Canopy Spread (cm)	No. of Leaves	Leaf Length (cm)	Leaf Width (cm)
1	Mauritius	98.87	111.47	37.40	71.60	5.13
2	Kew	93.60	123.00	46.20	78.00	5.20
3	MD-2	92.47	116.13	47.73	75.13	5.70
4	MTS	79.00	98.47	40.93	64.93	4.83
5	T-3	81.80	107.93	32.67	66.93	5.20
6	H-1	35.87	47.93	20.13	28.20	2.90
7	H-2	25.87	34.25	14.00	21.27	1.83
8	H-3	57.73	72.07	25.60	48.00	3.43
9	H-4	61.47	86.60	54.13	45.00	4.23
10	H-5	55.47	80.53	31.47	48.07	3.60
11	Amritha	59.00	78.27	3.67	50.80	3.91
	GM	67.33	87.00	34.63	54.36	4.18
	SEM	3.896	6.826	3.503	4.148	0.213
	CD (0.05)	11.495	20.137	10.335	12.238	0.632
	CV%	10.023	13.589	17.522	13.219	8.835

Table 14. Growth parameters of pineapple accessions 20 months after planting

No	Accessions	Plant Height (cm)	Canopy Spread (cm)	No. of Leaves	Leaf Length (cm)	Leaf Width (cm)
1	Mauritius	92.20	128.00	44.33	73.47	4.80
2	Kew	118.00	136.33	51.83	87.67	5.90
3	MD-2	98.93	124.93	52.67	78.47	5.30
4	MTS	65.87	112.47	38.87	59.93	4.33
5	T-3	92.03	116.27	37.57	67.87	5.47
6	H-1	37.87	60.13	27.97	30.40	4.10
7	H-2	29.87	44.77	19.17	24.20	2.40
8	H-3	69.07	90.73	32.90	54.60	4.57
9	H-4	74.07	87.40	61.13	46.80	4.63
10	H-5	66.87	78.17	35.67	55.27	3.40
11	Amritha	64.07	92.47	33.87	52.47	4.97
	GM	75.53	97.50	39.63	57.48	4.53
	SEM	4.568	5.461	3.134	3.397	0.198
	CD (0.05)	13.745	16.110	9.246	10.027	0.584
	CV%	10.759	9.701	13.698	10.255	7.569

1.3.1 Chilling & Shelf life Studies of Vazhakulam Pineapple (Mauritius)

Objective

To observe the changes in the fruit characters during chilling and shelf life for identifying export quality fruits

Technical Programme

For chilling studies fruits harvested from the field were refrigerated at 4^0C for 20 days. They were observed at an interval of every 5 days.

Shelf life studies were done by keeping the harvested fruits in room temperature for 15 days and were observed every 3 days interval.

Results

Tabulated observations of the yield characters, phytochemical characters and qualitative characters of different accessions of Mauritius variety is furnished below.

Table 15. Periodical changes in yield characters of Mauritius pineapple due to chilling

Days	Fruit+ crown wt. (g)	Crown wt. (g)	Fruit wt. (g)	Peel wt. (g)	Core wt. (g)	Juice wt. (g)	Pulp wt (g)
0	1482.00	152.00	1330.00	155.00	195.50	615.50	516.00
5	1543.00	146.00	1397.00	207.00	211.50	573.50	551.50
10	1486.50	207.00	1279.00	198.50	99.50	535.00	653.50
15	1494.00	153.00	1341.00	210.50	188.00	520.00	646.50
20	1614.00	135.00	1479.00	230.00	130.00	585.00	669.00

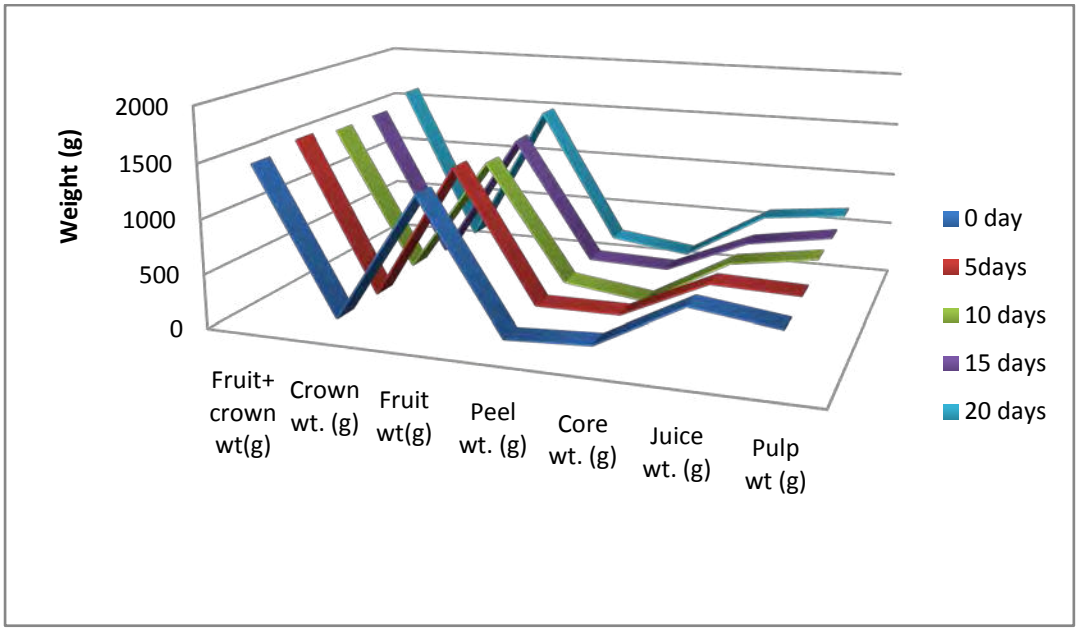


Fig. 11. Periodical changes in yield characters of Mauritius pineapple due to chilling

Table 16. Periodical changes in phytochemical characters of Mauritius pineapple due to chilling

Day	TSS (%)	pH	Acidity (%)	Reducing Sugar (%)	Non red. Sugar (%)	Total Sugar (%)	Ascorbic Acid (mg/100g)
0	17.00	3.54	0.88	2.80	13.00	15.8	70.58
5	20.20	3.20	0.75	2.34	11.79	14.13	61.89
10	23.00	3.30	0.74	3.85	13.20	17.05	73.35
15	14.80	3.20	0.95	2.12	12.45	14.57	68.34
20	12.00	3.20	0.94	2.04	13.80	15.84	73.14

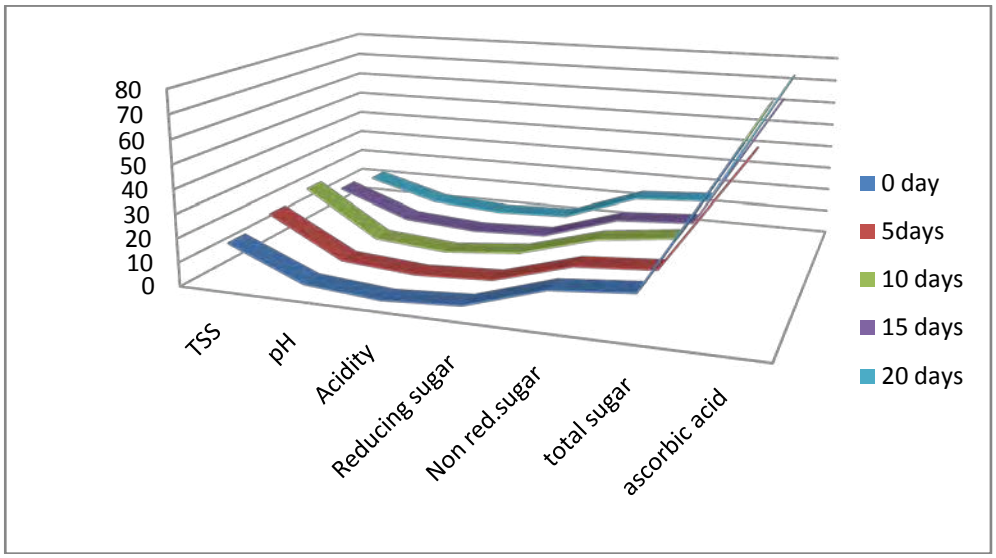


Fig. 12. Periodical changes in phytochemical characters of Mauritius pineapple due to chilling

After 0 day **After 5 days** **After10 days**

After15 days **After 20 days**

Fig. 13. Periodical changes in Mauritius due to chilling

Table 17. Periodical changes in qualitative characters of Mauritius pineapple due to chilling (0-9 scale)

Day	Taste	Colour	Size	Smell
0	5.5	5.0	6.0	5.0
5	4.0	5.0	6.5	4.0
10	5.8	6.0	7.0	5.5
15	4.0	5.0	6.0	4.0
20	3.5	4.0	5.0	3.0

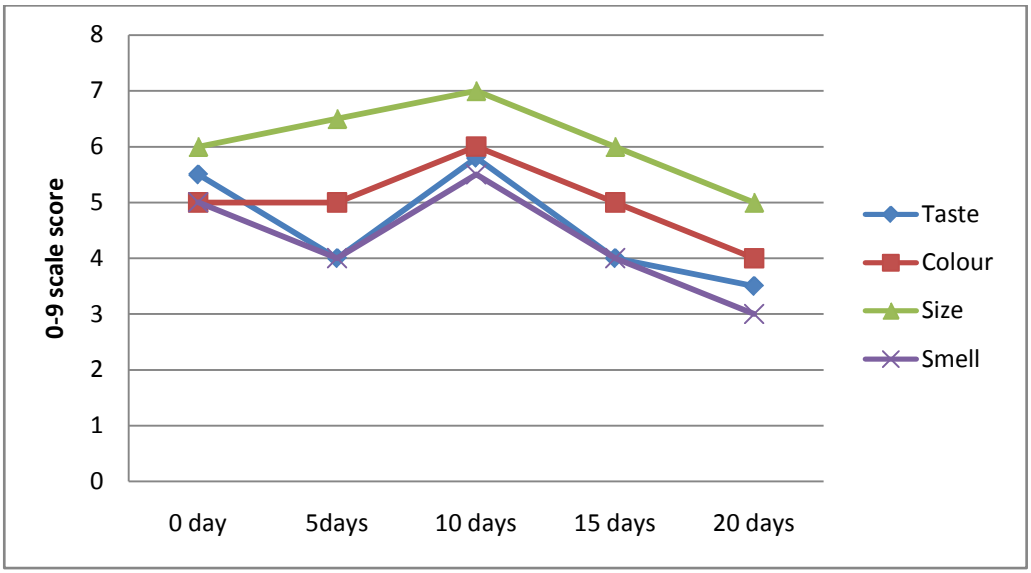


Fig. 14. Periodical changes in qualitative characters of Mauritius pineapple due to chilling (0-9 scale)

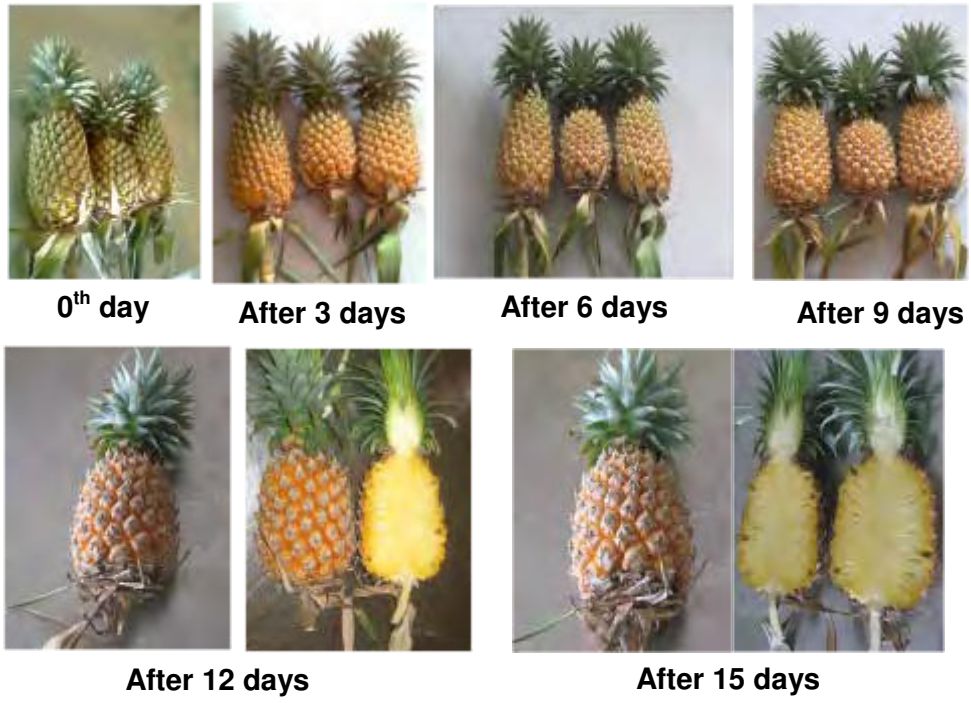


Fig. 15. Periodical changes in Mauritius during shelf life

Table 18. Periodical changes in yield characters of Mauritius pineapple during shelf life

Day	Fruit+ crown wt(g)	Crown wt. (g)	Fruit wt(g)	Peel wt. (g)	Core wt. (g)	Juice wt. (g)	Pulp wt (g)
0	1467.00	89.50	1377.50	120.50	200.00	656.00	401.00
3	1391.00	156.50	1234.50	190.00	124.00	524.00	396.50
6	1123.00	162.50	960.50	138.00	95.00	510.00	217.50
9	834.50	117.50	717.00	127.50	78.00	312.00	199.50
12	1292.00	162.50	1129.50	178.00	108.00	700.00	143.50
15	1345.00	146.00	1199.00	159.00	121.50	536.00	382.50

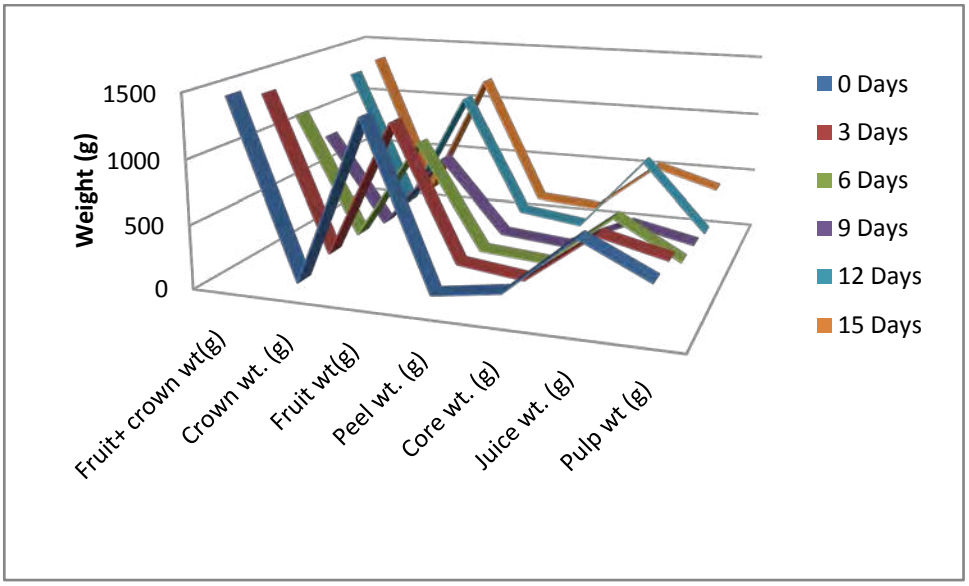


Fig. 16. Periodical changes in yield characters of Mauritius pineapple during shelf life

Table 19. Periodical changes in phytochemical characters of Mauritius pineapple during shelf life

Day	TSS (%)	pH	Acidity (%)	Reducing sugar (%)	Non red. Sugar (%)	Total sugar (%)	Ascorbic acid (mg/100g)
0	18.00	3.32	0.61	3.52	11.28	14.80	88.82
3	21.00	3.50	0.79	3.33	11.49	14.82	54.00
6	21.60	3.35	0.67	2.47	11.89	14.36	17.00
9	22.00	3.38	0.78	2.79	12.08	14.87	42.84
12	17.00	3.20	0.72	2.34	11.79	14.13	61.82
15	15.40	3.20	0.64	2.02	11.04	13.06	54.67

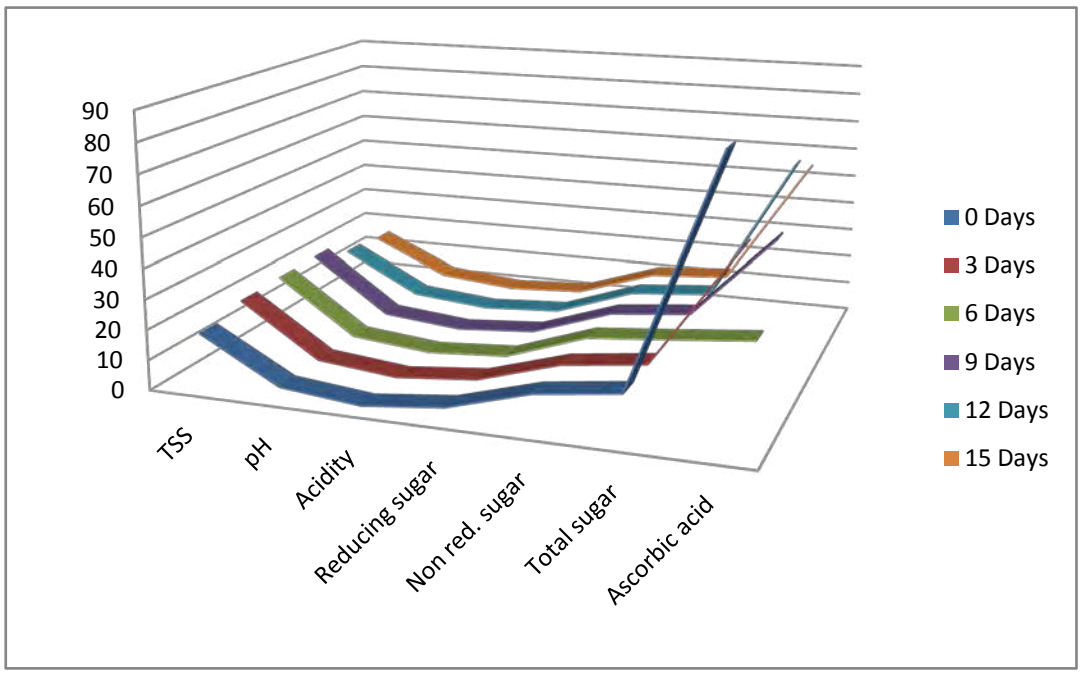


Fig. 17. Periodical changes in phytochemical characters of Mauritius pineapple during shelf life

Table 20. Periodical changes in qualitative characters of Mauritius pineapple during shelf life (0-9 scale)

Days	Taste	Colour	Size	Smell
0	5.50	5.00	6.00	5.00
3	5.50	5.00	5.50	5.00
6	5.50	6.00	5.00	6.00
9	5.50	6.00	4.00	6.00
12	4.50	6.00	5.50	5.00
15	4.00	5.00	5.00	4.00

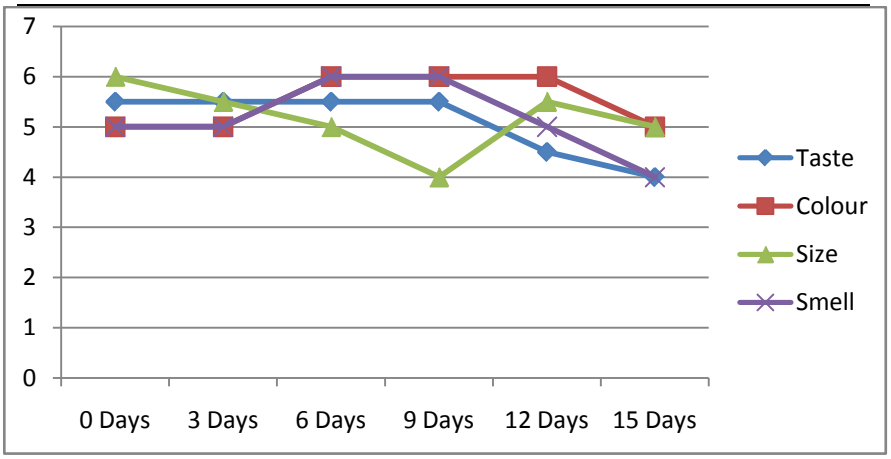


Fig. 18. Periodical changes in qualitative characters of Mauritius pineapple during shelf life

1.4 Plant Protection Studies

1.4.1 Virus Indexing

Virus Indexing is a standard protocol followed to facilitate production of quality planting material. Virus diagnosis and genetic fidelity testing should be done to ensure the tissue culture raised plants are true to the type and free from viruses or other fastidious pathogens. The advent of tissue culture/micropropagation technology has made it possible to produce genetic fidelity planting material to meet the domestic needs as well as international trade. However the tissue culture propagation, does not exclude the infection of viruses, viroids, phytoplasmas and bacteria unless the parental material and stock cultures used for tissue culture production are tested and maintained free from above mentioned pathogens. It is therefore essential that proper testing of the material should be done for the purpose of certification. In addition the laboratory producing tissue culture materials must require explicit infrastructure and expertise complying with prescribed standards.

Plant viruses as a rule can multiply only within the living cells; they are species or family specific and can never attack animals or vice versa. Temperate viruses remain embedded within the hosts' nucleic acid and are transmitted generation to generation just like genes of the host. Plant viruses vary in their mode of transmission. Aphids or other sap-suckers are the most common mode of transmission. Seed borne viruses have pollens and they are transmitted from male flower to female flowers. Nematodes can transmit certain viruses. Certain potato viruses are undergoing Sap transmission through cultivators, pruning, hands of workers, and clothing of workers.

Common methods for detecting plant viruses include: ELISA and RT-PCR. Grafting leaf from the suspected plant to an indicator plant can be used for virus detection as the viruses move from host plant and causes symptoms in the indicator plant. Sap Transmission method can also be used for virus detection. In this technique a drop of sap from the suspected plant is placed on an intact leaf of an indicator plant. Some grit is added to the sap and rubbed so as to scratch the leaf enabling virus transmission. Presence of virus can be confirmed if the indicator plants show symptoms of infections.

Objective:

To investigate presence of Pineapple Mealy bug Wilt Associated Viruses I and II in the pineapple samples by Enzyme Linked Immunosorbant Assay (ELISA).

Technical programme

Reagent preparation is detailed in the appendix 1.

Entire experimental procedure extended for two days. On the very first day one gram of the sample was weighed and grinded with 5ml coating buffer. This mixture was centrifuged at 5000rpm for 10 minutes. 100µl of sample supernatant and buffer (Coating buffer + 2% PVP)

was loaded on microtitre plates and incubated at 37ºC for one hour. After a three time wash with wash buffer, 200 µl of blocking buffer was added. This was again incubated at 37ºC for one hour. After a three time wash with the wash buffer, 200µl of primary antibody (Ab) solution was added and incubated at 4ºC for overnight.

On the second day incubated mixture was given a three times wash with the wash buffer and 200µl of secondary Ab solution was added. This reaction mixture was incubated at 37ºC for two hours and washed three times with the wash buffer. 200µl of substrate solution was added and was incubated at 37ºC in dark for 2 hours. 50µl of stop solution was added and read in Microplate reader.

Results

Readings obtained from Microplate reader are tabulated below.

Table 21. Readings obtained from Microplate reader

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank PM1 0.22		MTS PM1 - 0.002 Neg		MTS PM1 - 0.002 Neg		MTS PM1 0.00 Pos		MTS PM1 0.010 Pos			
B		MD-2 PM1 0.10 Pos		MD-2 PM1 - 0.024 Neg		NAPCL PM1 0.000 Pos		NAPC PM1 - 0.002 Neg		H-1 PM1 - 0.007 Neg		
C	H-1 PM1 - 0.003 neg		Kew PM1 - 0.013 Neg		Kew PM1 - 0.018 Neg							
D												
E	Blank PM2 - 0.002 B		MTS-PM2 - 0.013 Neg		MTS-PM2 0.00 Pos		MTS PM2 - 0.016 Neg		MTS PM2 - 0.004 Neg			
F		MD-2 PM-2 - 0.014 Neg		MD-2 PM-2 - 0.010 Neg		NAPCL PM-2 -0.026 Neg						
G	Kew PM-2 - 0.017 Neg		Kew PM-2 - 0.018 Neg		Kew PM-2 - 0.023 Neg		Kew PM-2 - 0.004 Neg					

Table 22. Interpretation of Microplate readings

Cut off	0.000		0.006	
MTS after L Heat treatment G				
MTS Heat L	+	-	+	-
Heat treatment	+	-	+	-
MD-2 L	+	-	+	-
MD-2 G	-	-	-	-
NAPC L	+	-	+	-
NAPC G	-	-	-	-
H-1 L	-	-	-	-
H-1 G	-	-	-	-
Kew L	-	-	-	-
Kew G	-	-	-	-

Fig. 19. Mealy bug affected pineapple

Fig. 20. Microscopic view (20x) of mealy bug

Fig. 21. Adding samples to the microtitre plates and Mircoplate Washer & Reader

1.4.2 Efforts for Contamination Reduction

1.4.2.1 Identification of Tissue Culture Contaminations

Although researchers follow essential protocols to maintain aseptic conditions, contamination is a serious problem affecting efficient tissue culture plant production. The microbial contamination causes the destruction of explants. The important contaminants are bacteria, fungi and viruses. Major causes of contamination are

- Infected explants taken for tissue culture
- Contaminated lab wares
- Presence of contaminants and spores in media preparation room, culture room and inoculation room
- Lack of proper sterilization of media and explants.

Objective

Identification of contaminations in tissue cultured explants using Gram staining and Lactophenol Cotton Blue staining techniques.

Technical programme

1. Gram staining

Gram staining is a differential staining technique based on the principle of difference in cell wall constitution of microorganisms. It is employed to differentiate gram positive from gram negative bacteria. Gram positive bacteria appear violet as it retains the primary stain crystal violet in the thick outer peptidoglycan layer. The gram negative bacteria appear pink, the colour of counter stain safranin as it loses the primary stain through thin peptidoglycan layer.

A thin film of bacterial culture was mounted on the glass slide which was air dried and heat fixed. The smear was flooded with crystal violet for one minute and was washed under slow running tap water. Slide was again flooded with Gram's iodine solution for one minute. Decolouriser (95% ethanol) was added and slide was kept for 10 seconds. Later on decolouriser was removed and counter stain safranin was added. Slide was kept for one minute. Afterward the slide was kept under running water. Finally slide was dried and one drop of oil was added prior to the observation under oil immersion microscope.

Results

The tissue culture contamination was gram stained and observed under oil immersion microscope found to be Gram negative rods. Further biochemical tests have to be done to identify the organism.

Fig. 22. Gram negative rods

2. Lactophenol cotton blue staining

Lactophenol cotton blue staining is used for the identification of fungus. Two methods are employed for Lactophenol cotton blue staining, namely, Tear mount method and Slide culture method.

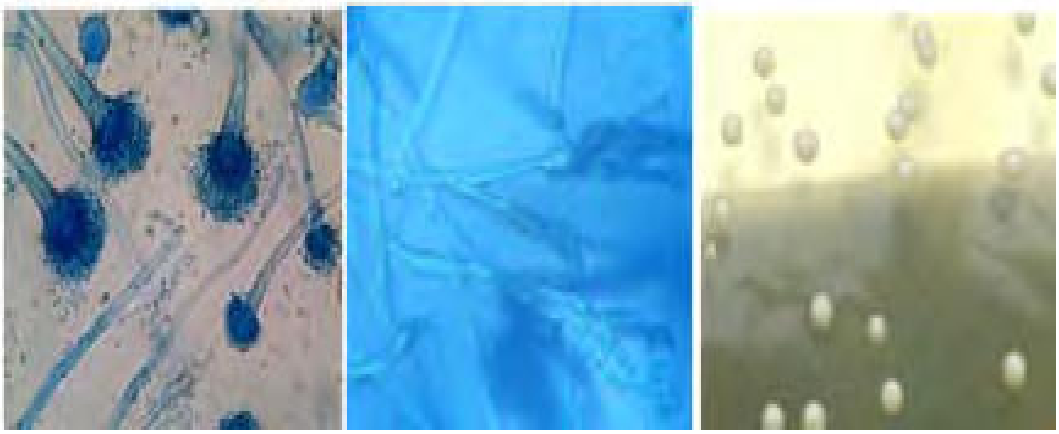
To perform tear mount method, one or two drops of Lactophenol cotton Blue stain was added to a clean slide. A little of the fungal mycelium was separated from the culture using a needle. Fungal mycelium was placed on the stain and teased with the needle so as to spread it. Cover glass was carefully placed taking extra caution to avoid air bubbles. Excess stain was removed using tissue paper and observed under 10X and 45X objectives of microscope.

Contaminations were also studied using Slide culture method for a double confirmation. To perform the same, slides were arranged over the v-shaped tube in a petriplate. 1cm×1cm square block of Sabouraud's dextrose agar was carefully placed on the centre of the glass slide and using an inoculation needle, fungal culture was neatly inoculated to 4 sides of the SDA block. Coverslip was placed with sterile foreceps and a moistened cotton in petriplate was kept for promoting the fungal growth. After two to three days incubation, agar block was carefully placed on a glass slide containing Lactophenol cotton blue staining. Block was later observed under 10x and 45x magnification of microscope.

Results

Aspergillus spp, Pencillium spp and Yeast spp were identified as tissue culture contaminations.

Fig. 23. Slide Culture Technique



Aspergillus spp ***Penicillium spp.*** ***Yeast spp.***

Fig. 24. Tissue Culture Contaminations Identified using Staining Techniques

Table 23. Observations of Lactophenol Cotton Blue Staining

Culture	Macroscopy	Microscopy		Identification
		Tease mount	Slide culture	
nf ba1* 17/8-15/9	Yellow colored fungal colonies Media colour changed to yellow	Branched hyphae Spherical spores Chain like arrangement Conidiophores Phialids present	Branched hyphae Spherical spores Chain like arrangemen Conidiophores septate mycelium phialids present	*Aspergillus spp.*
pa2** 25/3-16/9	Light bluish fungal growth(all over the media surface) Reverse is white	Broom like arrangement of spores on conidia Branched mycelium	Broom like arrangement of spores on conidia Phialids present Branched mycelium Spherical unstained spores Septate hyphae	*Penicillium spp.*
n-ba_k # 17/9-25/2	Dark bluish green Reverse is white	Branched hyphae, Conidia originates from conidiophores Phialids present	Branched hyphae Conidia originates from conidiophores Phialids present Unstained spores Septate hyphae	*Aspergillus spp.*
am pa_1.pa_2	Cream coloured colonie	Numerous oval shaped colonies	Numerous oval shaped colonies	*yeast spp.*

**PA2- specific medium used for plant tissue culture
#N- BA_K N- Nendran, BA_K - specific medium used for plant tissue culture
Am PA_1.PA_2- Amritha in PA_2 medium.
NF-BA1 –Nendran flower (explants), specific medium used for culture.

1.4.2.2 Identification of Pathogens from Diseased Plants

Objective

To Identify and isolate pathogens from diseased plant parts

Technical programme

The samples were collected from the field of pineapple research station, Vazhakulam. Infected parts were incised using knife and carried to lab in plastic bags. Sample analysis was done using Standard plate count method/ Serial dilution technique to determine the microbial load and to isolate individual microorganisms. Sterile water standards of 9 ml were prepared in test tubes. 1 g of the sample was weighed aseptically and was grounded using a sterile mortar and pestle which was later on dissolved in sterile 10 ml distilled water (1/10). Sample solution was serially diluted to 1/100000 dilution. (From sample tube 1 ml is serially diluted to all tubes and 1 ml is discarded from last tube) 0.1 ml sample from all the dilutions were plated in

nutrient agar and potato dextrose agar up to 1/10000 dilutions. Test tubes were incubated at 37º for 24-48 hours.

Result

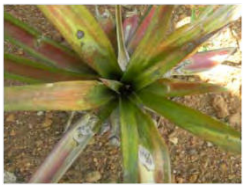
The SDA plates showed abundant growth. The colonies were observed under microscope by tear mount method to identify the pathogen. Macroscopic observation showed red coloured velvety growth on all plates. Microscopic observation proved the presence of branched mycelium, presence of sexual and asexual spores and Chlamydospores. Above observations confirmed the presence of Phytophthora spp. in the samples.

Table 24. Colony Count from Standard plate Count Method

Sample	Dilution	No. of colonies in SDA plates
Sample 1	1/100	TNTC*
	1/1000	TNTC
Sample 2	1/100	TNTC
	1/1000	TNTC
Sample 3	1/100	TNTC
	1/1000	TNTC

* Too numerous to count.

Table 25. Tabulated Summary of Diseased Samples

Sample	Plant part	Symptoms	Image
Sample 1	Pineapple leaf	Rotting (central portion of the stem) Top leaves turn brown Basal portion of leaves (signs of rotting with foul odour)	
Sample 2	Pineapple leaf	Large leaf spots Brownish or grayish patches yellowing of leaves Curling of leaf.	
Sample 3	Pineapple leaf	Water soaked lesions on the leaves Spots enlarge in size Gradually dry up.	

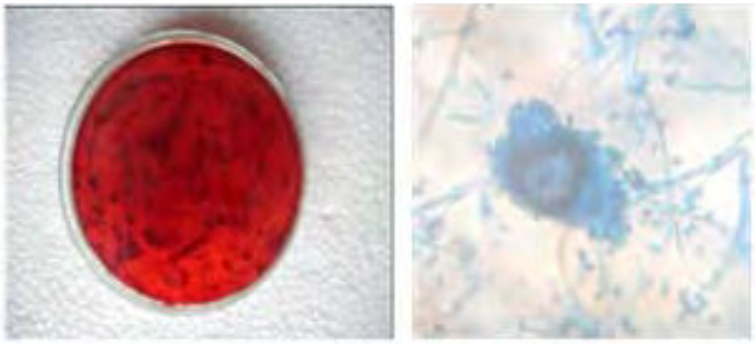


Fig. 25 . Macroscopic and Microscopic Observations of *Phytophthora spp* obtained from Pineapple leaves

1.4.2.3 Antifungal Sensitivity Tests

Objective

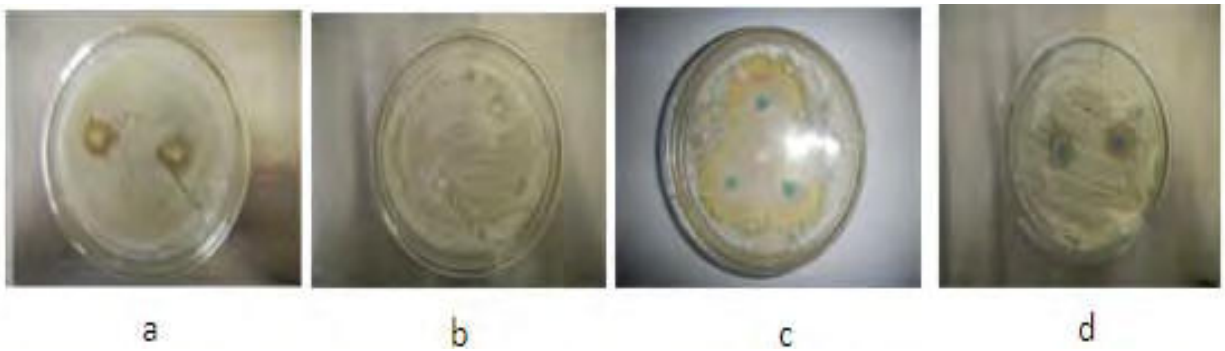
To determine the activity profile of various concentrations of Bavistin, Indofil, SAAF against isolated fungi.

Technical programme

Ten SDA plates were prepared in aseptic conditions. In sterile environment of LAF the fungal cultures- *Aspergillus* and *Penicillium* species were inoculated into the SDA plates using sterile cotton swabs. 2 mm wells were prepared on the SDA plates using sterile gel puncture and 0.1ml of various concentrations of the fungicides (Bavistin, Indofil, SAAF) were poured into each wells. The plates were incubated in an upright position at room temperature for 2-3 days. After incubation period, the sensitivity of fungi to the fungicides was measured.

Result

The important contaminating fungi in PTC laboratory were *Aspergillus* sp. and *Penicillium* sp. The major fungal contaminant was found to be *Aspergillus* sp. Indofil and SAAF are found to be resistant to *Aspergillus* spp. and *Penicillium* spp. Bavistin showed less effect on these fungal strains.



a. SAAF (0.5%) on *Aspergillus* sp. b. Bavistin (0.5%) on *Aspergillus* sp. c. Indofil (2%) on *Penicillium* sp. d. Indofil (1%) on *Aspergillus* sp.

Fig. 26. Response of *Aspergilus* and *Pencillium spp.* to different doses of fungicides

Table 26. Response of *Aspergilus* and *Pencillium spp.* to different doses of fungicides

Fungus	Fungicide	Concentration (%)	Zone of inhibition (mm)	Inference
Aspergillus spp.	Bavistin	2	2	-
		1	0	-
		0.5	0	-
	Indofil	2	13	+++
		1	8	++
		0.5	2	-
	SAAF	2	12	+++
		1	7	++
		0.5	0	-
Penicillium spp.	Bavistin	2	0.5	-
		1	0.4	-
		0.5	0	-
	Indofil	2	26	+++
		1	22	++
		0.5	19	+
	SAAF	2	30	+++
		1	26	++
		0.5	20	+

No change (-), Minimum (+), Medium (++), Maximum (+++)

1.4.2.4 Study on the Effects of Different Concentrations of Indofil and Saaf in Combination on Phytophthora spp.

Objective

To determine the combined effect of Saaf and Indofil on *Phytophthora spp*.

Technical programme

SDA media Plates were prepared and autoclaved to maintain sterile experimental conditions. Various combinations of fungicides used for analysis were prepared in distilled water. Fungicide concentrations used for testing were 0.5% SAAf + 0.5% Indofil, 0.5% Saaf + 1.0 % Indofil, 0.5% Saaf + 2.0% Indofil, 1% Saaf + 1.0% Indofil, 1% Saaf + 2% Indofil and 2% Saaf + 2% Indofil. Entire experiment sequences were carried out inside Laminar Air Flow Chamber. The *Phytophthora* fungal culture was swabbed onto the SDA plates using sterile cotton swab. 2 mm wells were prepared on the SDA plates using sterile gel puncture and 0.1ml of each concentration of the fungicides was poured into each wells. The plates were incubated in an upright position at room temperature for 2-3 days. After the incubation period the sensitivity of fungi to the fungicides was measured.

Result

0.5% Saaf and 2% Indofil combination was most effective against *Phytophthora* spp., whereas this species was found to be more resistant against the combination of 1% Saaf and 1% Indofil.

Table 27. Response of *Phytophthora* to different combinations of SAAF and Indofil

Concentration of Saaf + Indofil	Zone of inhibition (mm)	Response
0.5% Saaf+ 0.5% Indofil	30 mm	+ + +
0.5 % Saaf + 1% Indofil	23 mm	+ +
0.5% Saaf + 2% Indofil	34 mm	+ + +
1% Saaf + 1% Indofil	22 mm	+ +
1% Saaf + 2% Indofil	30 mm	+ + +
2% Saaf + 2% Indofil	25 mm	+ +

No change (-), Minimum (+), Medium (++), Maximum (+++)

Fig. 27 . Response of *Phytophthora* to different combinations of SAAF and Indofil

1.4.2.5 Plant Health Clinic Releases

Release 1. Pineapple Growth aberration

Crop and variety: Kew and T-3

Symptoms: The plant appearance is rosette like; leaves are narrower and very thickly packed.

Location: Experimental farm at NAPCL, Nadukkara, Muvattupuzha, Ernakulam District

Period : July 2011-April 2012

No. of plants affected: 2 plants

POP followed: KAU POP for Mauritius pineapple

Fig. 28. Diseased Kew

Release 2. Pineapple Fruit Rot

Crop & Variety: MD-2
Symptoms : Rotting of mature MD-2 fruit was observed. Fruit rot starts from the peduncle part of the fruit and spreads to whole part of the fruit. Fermenting smell is produced as the rotting progresses.
Location: PRS, experimental farm at NAPCL, Nadukkara, Muvattupuzha, Ernakulam District
Period: March – April 2012
No. of plants affected: About 6 fruits damaged. Problem is continuing.
POP followed: KAU POP for Mauritius pineapple is followed
Control measures taken: Indofil, 2.5 g/l sprayed initially, followed by Saaf, 2 g/l after one month.


MD-2 fruit rot initial stage


MD-2 fruit rot progressed


Fermented appearance


Longitudinal section

Fig. 29. Fruit rot of MD-2

Release 3. Pineapple leaf rot

Crop & Variety: MD-2
Symptoms: The leaf thickens and water soaked appearance. Foul smell. Leaf detaches from the plant.
Location: PRS, experimental farm at NAPCL, Nadukkara, Muvattupuzha, Ernakulam District
Period: April 2012
No. of plants affected: 1 plant
POP followed: KAU POP for Mauritius pineapple is followed
Control measures taken: Indofil, 2.5 g/l sprayed initially, followed by Saaf, 2 g/l after one month.

Fig. 30. Pineapple leaf rot

1.5 Molecular Studies

1.5.1 DNA Isolation for studying Diseased Plant Samples

Objective

To isolate the DNA from diseased plant samples

Technical programme

Samples taken: Kew, H-1(nursery), H-1(Field)

0.5gm of fresh leaf material was grounded using pre- chilled mortar & pestle in presence of 5ml extraction buffer & 50µl β –mercaptoethanol. The homogenate was transferred into a 15ml centrifuge tube and incubated the sample at 65ºC for 20 minutes with occasional mixing by gentle inversion. Added equal volume of chloroform: isoamyl alcohol (24:1) & mixed by inversion and incubated at 65ºC for 5 minutes. Centrifuged at 10,000 rpm for 15 minutes at 4ºC. Removed the aqueous phase with a wide – bore pipette, transferred to a clean tube and added 0.6 volume of chilled isopropanol & mixed by quick gentle inversion till DNA was precipitated. Kept at -20ºC for half an hour. Centrifuged at 10,000 rpm for 10 minutes at 4ºC. Discarded the supernatant & washed the DNA pellet in 70% ethanol. Centrifuged at 10,000 rpm for 5 minutes at 4ºC. Supernatant was discarded. Air dried the pellet, dissolved in 50µl sterilized distilled water. Stored at -20ºC.

Result

DNA was isolated, dissolved in 50µl sterile water and stored at -20ºC.

1.5.2 Amplification of Plant DNA using PCR Technique

Polymerase Chain Reaction is widely held as one of the most important inventions of the 20th century in molecular biology. Small amounts of the genetic material can now be amplified to be able to identify, manipulate DNA, detect infectious organisms, detect genetic variations, including mutations, in plant genes and numerous other tasks.

PCR involves the following three steps: Denaturation, Annealing and Extension. First, the genetic material is denatured, converting the double stranded DNA molecules to single strands. The primers are then annealed to the complementary regions of the single stranded molecules. In the third step, they are extended by the action of the DNA polymerase. All these steps are temperature sensitive and the common choice of temperatures is 94^{o}C, 60^{o}C and 70^{o}C respectively. Good primer design is essential for successful reactions

Objective

To amplify the isolated DNA from pineapple by using PCR Technique

Technical programme

Here the amplification is done by using a sample DNA provided with PCR kit and the DNA isolated from pineapple.

Materials: PCR kit, thermal cycler, sample DNA, Micropipette.

Reaction mixture: A

1. Sterile water - 19 µl
2. 10X assay buffer- 2.5 µl
3. 10 µm dNTP- 1.5 µl
4. Forward primer-0.5 µl
5. Reverse primer- 0.5 µl
6. Template DNA- 10X diluted- 0.5 µl
7. Taq. DNA polymerase- 0.5 µl

Total reaction mixture- 25 µl

Reaction mixture: B: - sample DNA provided with the kit.

1. Sterile water - 38 µl
2. 10x assay buffer- 5 µl
3. 10 µm dntp- 3 µl
4. Forward primer-1 µl
5. Reverse primer- 1 µl
6. Template dna- 10x diluted- 1 µl
7. Taq. Dna polymerase- 1 µl

Total reaction mixture- 50 µl

Reaction mixture was kept in ice bath.

Reaction condition

Initial denaturation	- 94ºC	1 minute
Denaturation	- 94ºC	2 minute
Annealing	- 48 ºC	30 seconds
Extension	- 72 ºC	1 min
Final extension	- 72 ºC	2 min

Lid temperature 105 ºC , hold temperature 4 ºC → for 30 cycles

1.5.3 Agarose Gel Electrophoresis

After PCR amplification, 5 µl gel loading buffer was added to the reaction tubes and mixed well. 15 µl samples were loaded in 0.8% agarose gel prepared in 1X TAE buffer. The age set up was done and run for approximately 2 hours. (Applied Voltage: - 80 volts DC). After AGE was completed, the gel is observed in UV Transilluminator.

Results

DNA bands were observed as orange bands

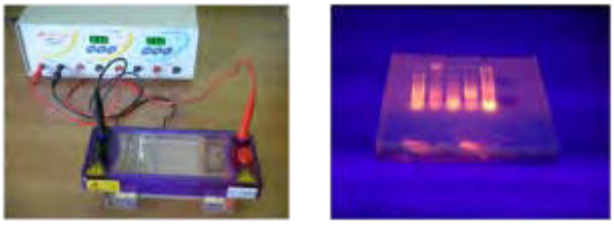


Fig. 31. Electrophoresis Unit and DNA bands obtained in Gel Doc

2 RESEARCH ON PASSION FRUIT

Passion fruit is a woody, perennial vine that bears delicious fruits and occurs in purple- and yellow-fruited forms *(Passiflora edulis* Sims f. *edulis* and *P. edulis* f. *flavicarpa)* known as purple and yellow passion fruits. The plants have a weak tap root and extensive ivory-colored lateral roots. The stem is usually solitary, up to 7 cm in basal diameter, extends 5 to 10 m or more into the crowns of trees, and is covered by a thin, flaky, light brown bark. The stem-wood is light and brittle. The twigs are yellow-green, turning brown, and support themselves on vegetation by means of tendrils that arise at the leaf axils. The leaves are alternate, green to yellow-green, three-lobed (on mature plants) with serrate edges.

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Fig. 32. Passion fruit- yellow Passion fruit-Purple

2.1 Micropropagation of Passion Fruit

Micropropagation studies of Passion fruits are in the initial stages and the standardization of the fresh inoculation of explants is completed. Available results are furnished below.

Explant source: Yellow and Purple Passion fruit seedlings in the Green house of Pineapple Research Station, Vazhakulam.

Objective

To standardize the inoculation medium for fresh inoculation of passion fruit explants

Technical programme

For surface sterilization, the soil exposed portions of the plants were chopped off. The explants were kept in running water for 30 minutes. Pril wash was given to clean the explants for 20 minutes. They were kept in running water till the complete removal of soap. Bavistin (0.1%) and Indofil (0.1%) was prepared in combination. The explants were dipped in the above solution for 30 minutes. The explants were washed in distilled water and treated with 70% ethanol. In the LAF a 70% ethanol wash was given to the explants. They were dipped in 0.1% $HgCl_2$ (w/v) for 6 minutes followed by 5 rinses in sterile distilled water. Nodes and leaves were cut and dipped in antibiotic (Gentamycin 1ml/l) solution and inoculated into the medium.

The explants were taken out of the beaker keeping it inside the laminar air flow cabinet using sterile forceps. They were cut to get maximum number of nodes and were immersed in sterile jam bottle. They were dipped in 70% alcohol for 2 minutes. 0.1% $HgCl_2$ was poured into the jam bottle for 3 minutes. They were washed with sterile distilled water 4-5 times to remove the excess $HgCl_2$. They were transferred to another sterile jam bottle containing 1ml/l Gentamycin. The explants were given a dip in antibiotic solution. Using sterile forceps the explants were inoculated into the medium kept in jam bottle. The bottles were carefully tightened and properly labeled before transferring to the incubation room at 25-27^{o}C.

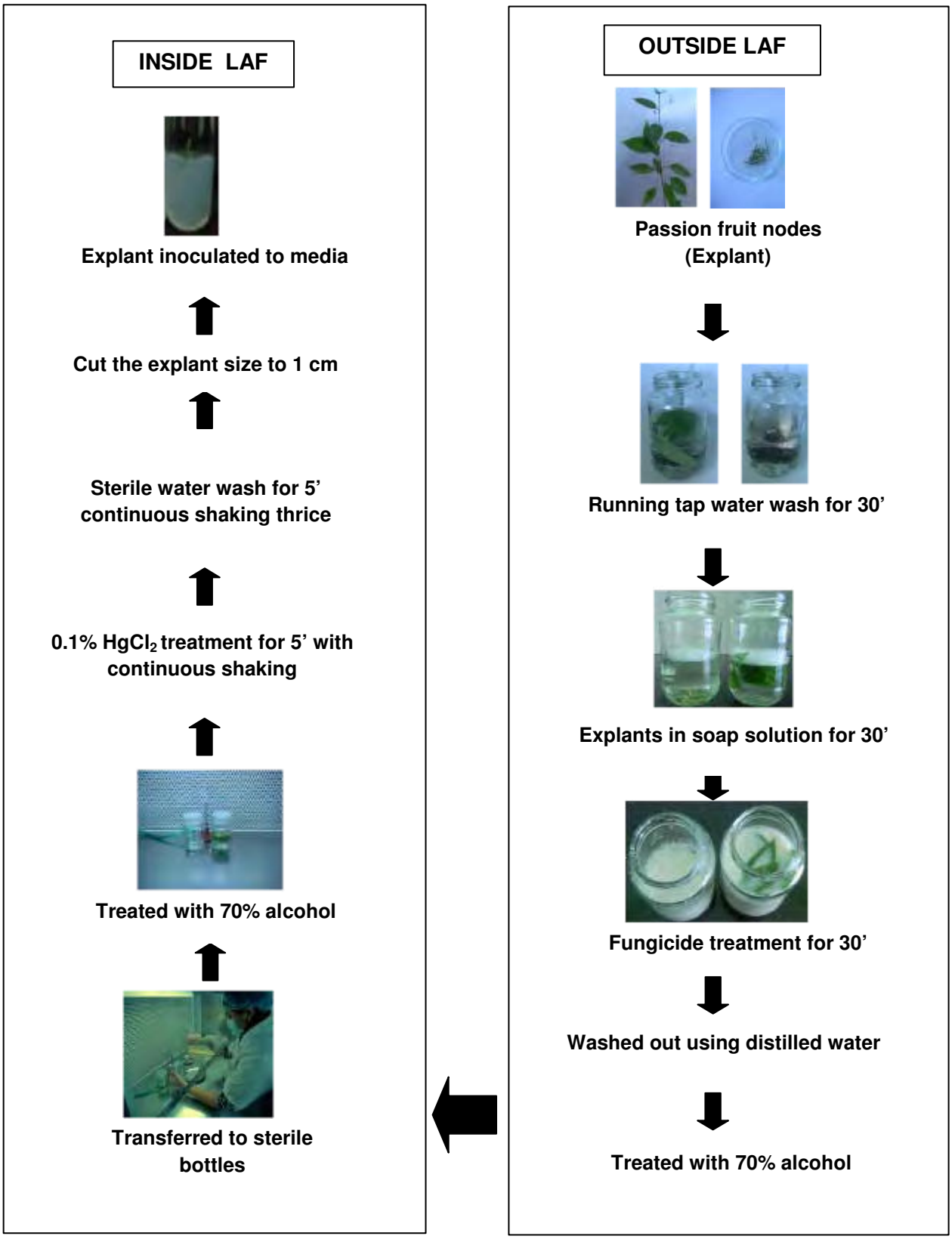

Fig. 33. Steps in fresh inoculation of Passion fruit

Fig. 34. Stages in initiation of Passion fruit Tissue culture

Table 28. Periodical changes in Passion Fruit nodes in different media

Medium	After 7 Days	After 14 Days	After 21 Days	Response
MS + 2.3 mg/l BA + 1.2 mg/l IBA + 1 g/l activated charcoal	Green	Green	Green	-
MS + 1 g/l activated charcoal	Green	Green	Green	-
MS + 4 mg/l BA	Green	Green	bulging	+
MS + 3 mg/l BA	Green	Green	bulging	+
MS + 2.5 mg/l BA	Green	bulging	bulging	+ +
MS + 2 mg/l BA	Shoot growth from node	Multiple shoots developed	Multiple shoots increased with callusing	+ + +
MS + 1 mg/l BA	Green	Green	Green	-

No Response (-); Minimum Response (+); Medium Response (+ +); Maximum Response (+ + +)

2.2 Evaluation of Passion Fruit types for the plains of Kerala

Objective

To select a passion fruit variety suitable for the plains of Kerala state.

Technical programme

The project was started in the year 2003, as a collaborative project with NAPCL, Nadukara. It was continued for 3 years as collaborative project and later it was taken up as a KAU project. Passion fruit accessions were collected from different parts of Kerala and various locations outside the state also. Yellow, purple and giant types are available in the collections. The accessions were evaluated continuously all the years and the plants are in a declining stage now. During the year 2011-12, 7 plants, set fruits and they were evaluated for yield, biochemical and qualitative characters.

Experimental programme followed for the entire passion fruit entries can be broadly classified as analysis of yield characters, phytochemical characters and qualitative characters. Yield character studies included detection of number of fruits under each accession numbers, calculation of fruit weight, rind weight, pulp weight, seed weight and juice weight. Phytochemical analysis quantified the TSS, pH, acidity, ascorbic acid, reducing sugars, non-reducing sugars and total sugar. Taste, colour, size and aroma of the fruits were scored in 0-9 scale qualitatively.

Result

Data on entire passion fruit accessions of the year 2010-11 are tabulated below. Yield characters, phytochemical characters and qualitative characters of individual passion fruits were observed. Passion fruit with accession number 88 possessed maximum rind weight and juice weight followed by accession number 57. Pulp weight was highest for the accession no. 55 followed by 57. Highest seed weight was shown by passion fruit with accession number 57.

Coming to the phytochemical characters, every passion fruit showed same PH. Passion fruit with accession number 86 had the highest acidity and reducing sugar content. Passion fruit with accession number 32 had lowest acid content. Accession number 57 showed highest amount of Ascorbic acid, non reducing sugar and total sugar which was followed by accession number 88.

Qualitative characters analysis confirmed the passion fruit with accession no. 88 had good taste, colour, size and aroma followed by accession number 86.

Table 29. Yield Characters of Passion Fruits with Different Accessions

Plant No.	No. of Fruits	Fruit weight(g)	Rind weight(g)	Pulp weight(g)	Seed weight(g)	Juice weight(g)
32	3.00	296.50	159.50	127.50	12.50	84.50
45	16.00	1264.00	724.00	656.00	27.00	528.50
55	25.00	1962.00	896.00	757.50	60.80	485.50
57	28.00	1844.50	961.50	751.00	77.00	555.50
66	3.00	166.00	112.50	74.50	7.50	56.50
86	6.00	430.00	236.50	158.50	12.50	148.50
88	27.00	672.50	977.00	671.50	64.00	754.50

Table 30. Phytochemical Characters of Passion Fruits with Different Accessions

Plant No.	TSS (%)	Acidity (%)	Ascorbic Acid(mg/100g)	Red. Sugar (%)	Non red. Sugar (%)	Total sugar (%)
32	20.6	2.39	34.08	4.40	11.20	15.60
45	24.0	3.17	47.45	4.18	11.33	15.51
55	23.6	2.76	44.73	4.02	10.79	14.81
57	23.4	2.85	59.92	4.57	12.03	16.60
66	23.6	3.14	38.02	4.40	10.20	14.60
86	22.8	3.62	43.74	5.60	9.60	15.20
88	23.4	2.98	55.60	4.55	11.60	16.15

Table 31. Qualitative characters of passion fruit accessions (0-9scale)

Plant No.	Taste	Colour	Size	Aroma
32	3.00	5.00	5.00	5.00
45	5.00	5.30	4.50	5.00
55	4.20	5.00	6.00	5.00
57	4.20	5.20	4.80	5.00
66	5.00	5.00	4.50	6.00
86	5.00	5.50	6.00	6.00
88	5.00	5.60	6.30	5.60

3. RESEARCH ON BANANA

3.1 Micropropogation of Banana

We are mainly focusing on the tissue culture studies of four varieties of banana namely Nendran, Njali Poovan, Robusta and Red banana. Another variety named Grandnaine is also studied here. Explants used for tissue culture were shoot tips and inflorescence of banana. Micropropogation of banana includes four major stages: *Initiation (Fresh inoculation), Mass Production (Multiplication), Rooting and Hardening (Plant out).* Multiplication stage of banana involved media change and subculture procedures.

Stage 1. (a) Fresh Inoculation of Banana shoot tip (sucker)

Objective

To freshly inoculate banana shoot tip (sucker) and standardize the ideal inoculation medium for banana shoot tip (sucker).

Technical programme

Explant used for fresh inoculation was banana suckers. Suckers were washed thoroughly in tap water, roots and leaf sheaths were removed, and the basal portion of the corm was cut and trimmed to a size of 12 x 12 x 15mm. Explants were kept under running tap water for 30 minutes, soaked in teepol (detergent) for 30 minutes and shaked continuously, washed with distilled water to remove the teepol particles, treated with fungicide for 30 minutes and washed with distilled water to remove fungicide. They were then transferred to laminar air flow chamber for surface sterilization. Inside the laminar air flow chamber, the explants were treated with 70% ethanol (2 minutes) and then with 0.1% Mercuric chloride for 5 minutes followed by three rinsing of 5 minutes each with sterilized distilled water. The explants were trimmed to a final size of 8x8x10mm in sterile conditions of the inoculation chamber, and inoculated on culture medium then incubated at 25±2^{o}C dark for 21 days.

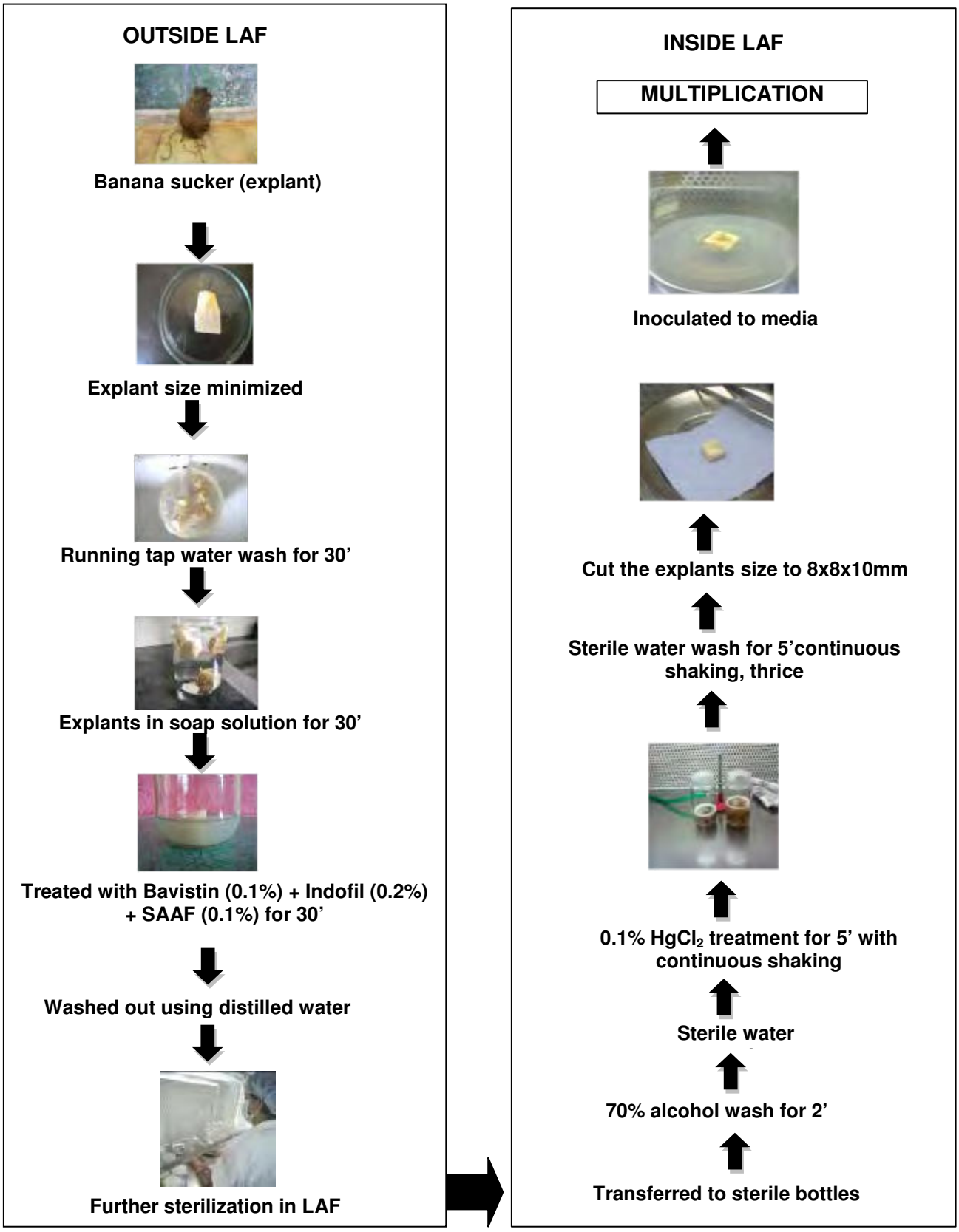

Fig. 35. Fresh Inoculation of Banana shoot tip

Table 32. Standardization of Media for fresh Inoculation of Banana shoot tip (sucker)

Banana Variety	Medium	After 7 days	After 14 days	After 21 days	Response
Nendran	MS+2.5mg/l BA+0.5mg/l kinetin	bulging	Increased bulging	1 bud	+ +
	MS+3mg/l BA	bulging	Increased bulging	3.5 buds	+ + +
	MS+5mg/l BA	bulging	bulging	Elongation bulging	+
	MS+3mg/l BA+0.2mg/l NAA	No change	bulging	bulging	+
	MS+2.5mg/lBA+1.3mg/lIAA+75mg/lAscorbic acid+75mg/lCitric acid	No change	No change	No change	-
Poovan	MS+2.5mg/l BA+0.5mg/l kinetin,	bulging	bulging	Bulging&1 bud	+ +
	MS+3mg/l BA	bulging	bulging	Bulging 1.5 multiple buds	+ + +
Red Banana	MS+3mg/l BA	bulging	bulging	Bulging & 3 multiple buds	+ + +
	MS+5mg/l BA	bulging	bulging	Elongation & bulging	+ +
	MS+3mg/l BA+0.2mg/l NAA	No change	bulging	bulging	+
	MS+2.5mg/lBA+1.3mg/lIAA+75mg/lAscorbic acid+75mg/l Citric acid	No change	No change	No change	-
Robusta	MS+3mg/l BA	bulging	bulging	Bulging & 2 multiple buds	+ + +

Result

The fresh inoculated banana shoot tip (sucker) cultures were observed for a cycle of 21 days. After 7th days bulging was started along with elongation of central bud. Within 14 days buds were started sprouting. 21 days were required to increase the bud number from 0 to 3.5. No callus formation was observed in the medium. The same medium was used for media change of the cultures for increase in buds number

Stage 1.b. Fresh Inoculation of Banana Inflorescence

Objective

To standardize fresh inoculation medium of Nendran, Robesta and Njali Poovan inflorescences

Technical programme

Explant Source: Nendran Inflorescence was obtained from farmers and Njali Poovan inflorescence was collected from healthy plants after the formation of all the female flowers. The bracts with the male flowers were removed until they become too small (3 cm in length).

For surface sterilization the plant parts (Explants) used for fresh inoculation were kept under running tap water for 30 minutes, soaked in teepol (detergent) for 30 minutes and shaked continuously, treated with fungicide (bavastin 0.1%, Indofil 0.1% and SAAF 0.05%) for 30 minutes and washed with distilled water to remove fungicide. They were then transferred to laminar air flow chamber for surface sterilization.

Inside the laminar air flow chamber, the explants were treated with 70% ethanol (2 minutes) and then with 0.1% Mercuric chloride for 5 minutes followed by three rinsing of 5 minutes each with sterilized distilled water. The explants were trimmed to a final size of 8x8x10 mm in sterile conditions of the inoculation chamber, and inoculated on culture medium. Nendran shoot tips were inoculated to both MS+ 3mg/l BA and MS+2.5 mg/l BA+ 0.5mg/l Kinetin.

Result

The fresh inoculated banana inflorescence cultures were observed for a cycle of 21 days. After 7 days bulging started and within 14 days buds sprouted. 21 days were required to increase the bud number from 0 to 3. No callus formation was observed in the medium. Also the same medium was used for media change of the cultures for increase in buds number.

Table 33. Standardization of Media for fresh Inoculation of Banana Inflorescence

Banana Variety	Medium	After 21 Days	Response
Nendran	MS + 3mg/l BA	2.5 Buds	+ + +
	MS + 2.5mg/l BA + 0.5 Kinetin	1 Bud	+ +
Robusta	MS + 3mg/l BA	2.5 Buds	+ +
	MS + 2.5mg/l BA + 0.5 Kinetin	3 Buds	+ + +
Njali Poovan	MS + 3mg/BA	Bulging	+
	MS + 2.5mg/l BA + 0.5 Kinetin	Bulging	+

Minimum Response (+); Medium Response (+ +); Maximum Response (+ + +)

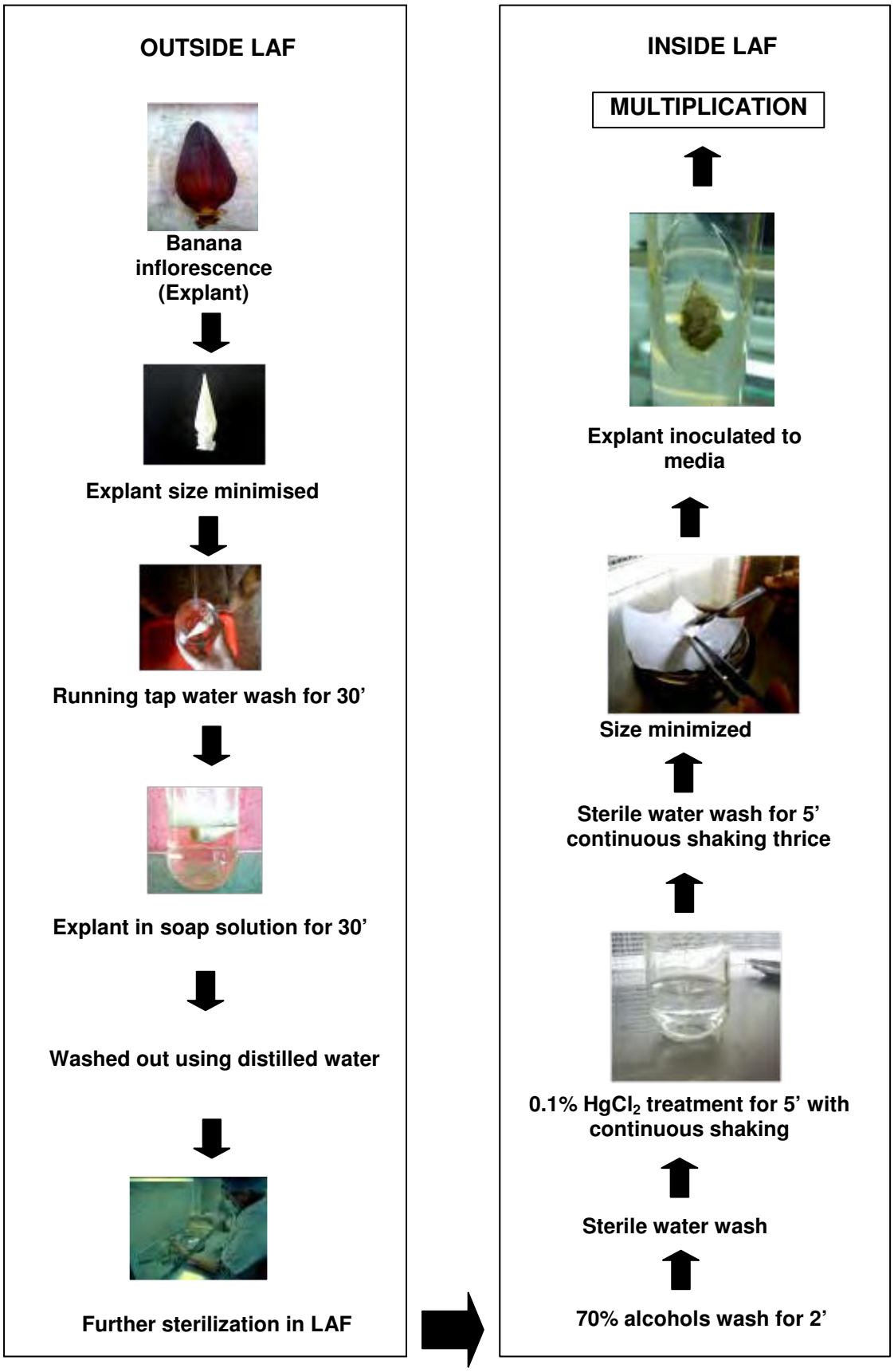

Fig. 36. Fresh inoculation of banana inflorescence

Stage 2. Multiplication

2.1 Media change of Banana shoot tip (sucker)

Objective

To media change banana shoot tip (sucker)

Technical Programme

Responding shoot tip explants were transferred to fresh medium after 21 days of incubation in the dark condition and after 3-4 days in dark they were transferred to photoperiodic conditions 16h:8h. It was essential to change the medium in every 21 days; otherwise the phenols formed will inhibit the growth. The inflorescence tips were carefully removed from the medium, basal portion was removed and inoculated to fresh medium. After 90-120 days, initiated multiple shoots were divided into clusters of 4-6 shoots and sub cultured to the multiplication medium for multiplication. This process of division into clusters and subculture is repeated for five generations.

Result

During 1st media change the banana shoot tip (sucker) cultures increased its multiple bud formation. After 2nd media change the buds colour changed to green. Number of buds increased after 3rd media change.

Table 34. Standardization of Media change for fresh Inoculation of Banana shoot tip (sucker)

Banana Variety	Medium	1st media Change	2nd Media Change	3rd Media Change	Response
Nendran	MS+2.5mg/ l BA+0.5mg/l kinetin	Multiple bud formation	Dark green colour buds	Number of buds increased	+ +
	MS+3mg/l BA	Multiple bud formation& slight green colour buds	No of multiple buds Increased dark green colour buds	Increase in multiple shoots	+ + +
Poovan	MS+2.5 mg/l BA+0.5mg/l kinetin,	Delay in Multiple bud formation	No of multiple buds increased Dark green colour buds	Increase in multiple shoots	+ +
	MS+3mg/l BA	Multiple bud formation	No of multiple buds increased	Increase in multiple shoots	+ + +
Red Banana	MS+3mg/l BA	Multiple bud formation Slight green colour buds	Increase in multiple buds Dark green colour buds	Increase in multiple shoots	+ + +
Robesta	MS+3mg/l BA	Multiple bud formation	Dark green colour buds	Increase in multiple shoots	+ + +

Minimum Response (+); Medium Response (+ +); Maximum Response (+ + +)

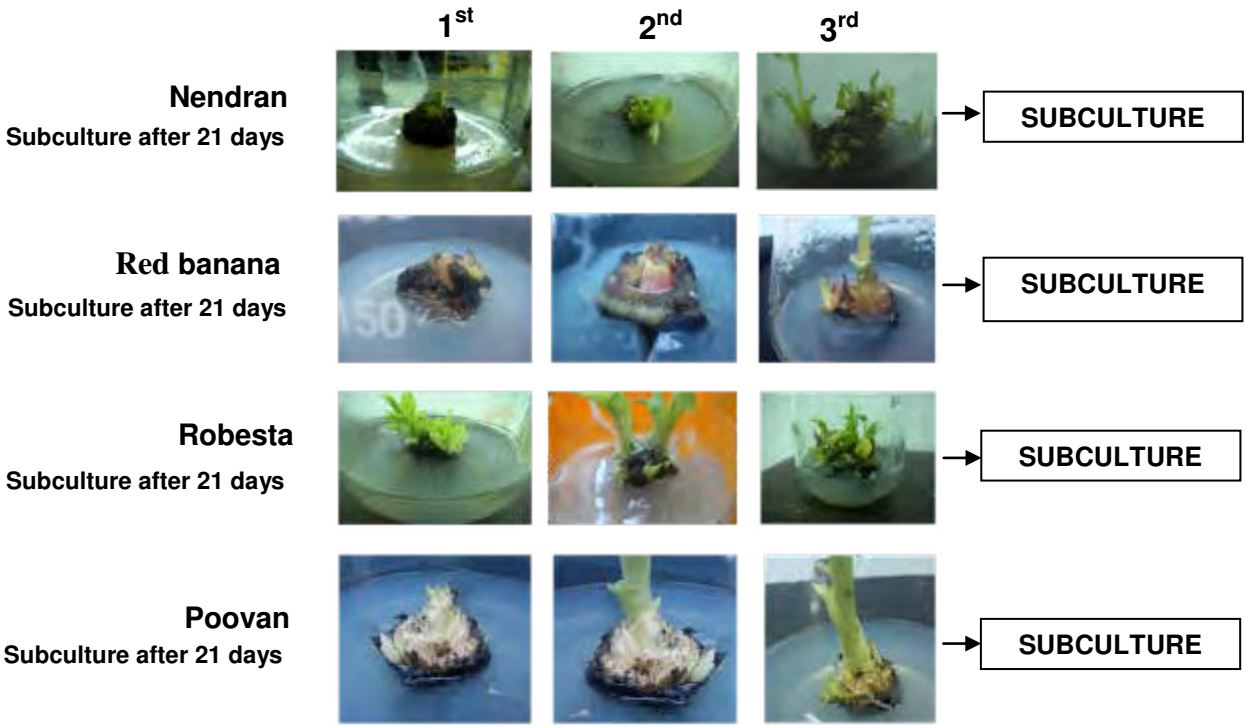

Fig. 37. Media change of Banana Shoot Tip

2.2 Media change of Banana Inflorescence

Objective

To standardize medium for media change of banana inflorescence

Technical Programme

Responding shoot tip explants were transferred to fresh medium after 21 days of incubation in the dark condition and after 3-4 days in dark they were transferred to photoperiodic conditions 16h:8h. It was essential to change the medium in every 21 days; otherwise the phenols formed will inhibit the growth. The inflorescence tips were carefully removed from the medium basal portion was cut and inoculated to fresh medium. After 90-120 days, initiated multiple shoots were divided into clusters of 4-6 shoots and sub cultured to the multiplication medium for multiplication. This process of division into clusters and subculture is repeated for five generations.

Result

MS+3mg/l BA medium was found commonly effective for all the banana inflorescences. After 3rd media change increase in multiple buds was observed in the medium.

Table 35. Tabulated Results of Media change of Banana Inflorescence

Banana Variety	Medium	1st media change	2nd media change	3rd media change	Response
Nendran	MS+3mg/l BA	Multiple bud formation & Slight green colour buds	Increase in multiple bud formation Slight green colour buds	Increase in multiple shoot	+ + +
	MS+2.5 mg/l BA+0.5mg/l kinetin	Slight green colour buds	Multiple bud formation Dark green colour buds	Slight Increase in Multiple shoot	+ +
Robusta	MS+3mg/l BA	Multiple bud formation	Increase in Multiple bud formation Dark green colour buds	Slight Increase in Multiple shoot	+ +
	MS+2.5 mg/l BA+0.5mg/l kinetin	Multiple bud formation & Slight green colour buds	Multiple bud formation Slight green colour buds	Increase in multiple shoot	+ + +
Njali poovan	MS+3mg/l BA	Multiple bud formation	Increase in Multiple bud formation Dark green colour buds	Increase in multiple shoot	+ + +
	MS+2.5 mg/l BA+0.5mg/l kinetin	Delay in multiple bud formation.	Multiple bud formation	Colour turn to dark green	+ +

Minimum Response (+); Medium Response (+ +); Maximum Response (+ + +)

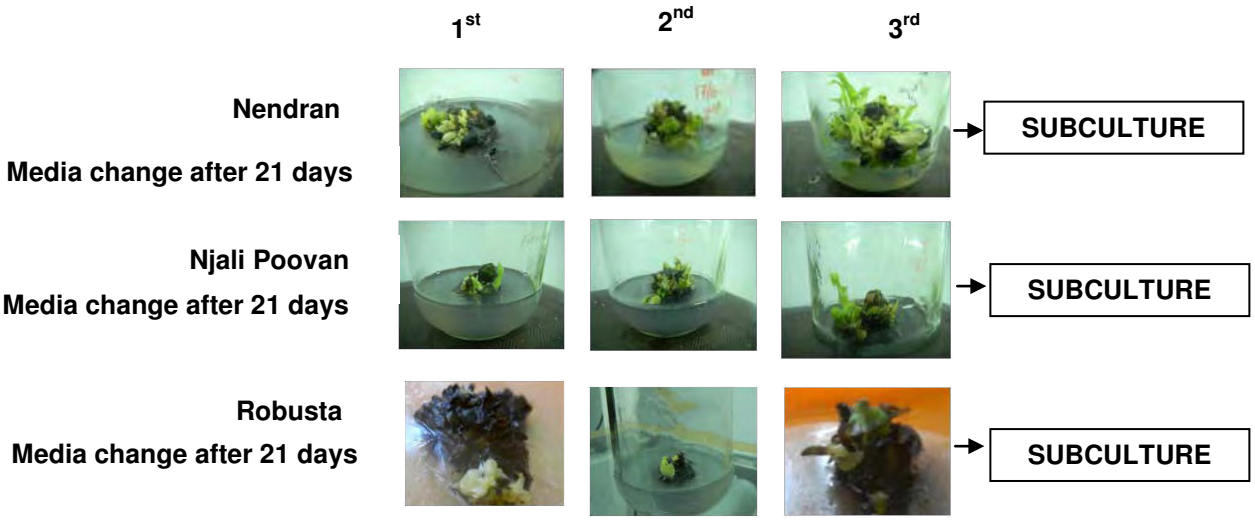

Fig. 38 . Media Change of Banana Inflorescence

2.3 Subculture of Banana shoot tip (sucker)

Objective

To standardize the medium for subculture of banana shoot tip (sucker).

Technical Programme

MS+3mg/lBA medium was not effective for multiplication. Hence Banana shoot tip (sucker) cultures were sub cultured to MS+3mg/lIBA+0.5mg/lBA medium for increase in buds number and callus formation. This stage continued till 6th subculture. After enough cultures were obtained they were subcultured to rooting medium for root formation.

Result

MS+3mg/l IBA+1.5mg/l BA medium was found effective for all the banana shoot tips except for poovan. Increased bud formation up to 16 buds in case of red banana was observed after 3rd subculture. MS+2mg/l BA medium was found effective only in case of poovan.

Table 36. Shoot production in sequential subculture of banana shoot tip (sucker)

Banana Variety	Medium	1st subculture	2nd subculture	3rd subculture	Response
Nendran	MS+3mg/l IBA+1.5mg/l BA(a)	3 buds	7.5 buds	10 buds	+ + +
Poovan	MS+3mg/l IBA+1.5mg/l BA(a)	2 buds shoot elongation	3.5 buds shoot elongation	6 buds shoot elongation	+ +
	MS+2mg/l BA(b)	5 buds	9.5 buds	14 buds	+ + +
Red Banana	MS+3mg/l IBA+1.5mg/l BA(a)	7 buds	13.5 buds	16 buds	+ + +
Robusta	MS+3mg/l IBA+1.5mg/l BA(a)	6.5 buds	12 buds	14 buds	+ + +

Minimum Response (+); Medium Response (+ +); Maximum Response (+ + +)

2.4 Subculture of Banana Inflorescence

Objective

To standardize the medium for subculture of banana Inflorescence

Technical Programme

MS+3mg/lBA medium was not effective for multiplication. Hence Banana inflorescence cultures were subcultured to MS+3mg/lIBA+0.5mg/lBA medium for increase in buds number and callus formation. This stage was continued till 6th subculture. After enough cultures were obtained they were sub cultured to rooting medium for root formation

Result

MS+3mg/l IBA+1.5mg/l BA medium was found effective exclusively for banana inflorescence. 22 shoots at 3rd subculture was observed to a maximum in Nendran.

Table 37. Shoot production in sequential subculture of banana inflorescence

Banana Variety	Medium	1st subculture	2nd subculture	3rd subculture	Response
Nendran	MS+3mg/l IBA+1.5mg/l BA	6 shoots	17 shoots	22 shoots	+ + +
Njali Poovan	MS+3mg/l IBA+1.5mg/l BA	4 shoots	10 shoots	17 shoots	+ + +
Robesta	MS+3mg/l IBA+1.5mg/l BA	8 shoots	14 shoots	16 shoots	+ + +

Minimum Response (+); Medium Response (+ +); Maximum Response (+ + +)

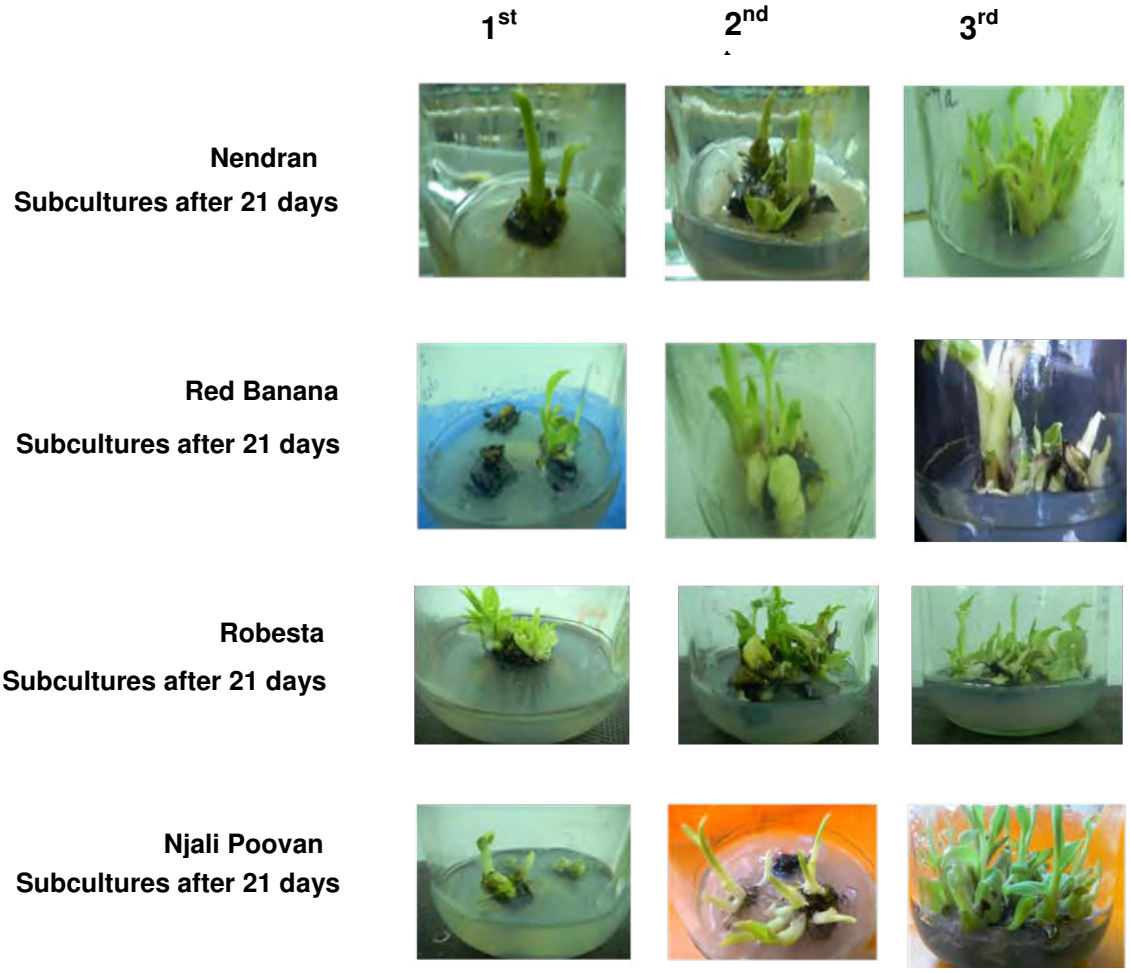

Fig. 39. Shoot production in sequential subculture of banana shoot tip (sucker)

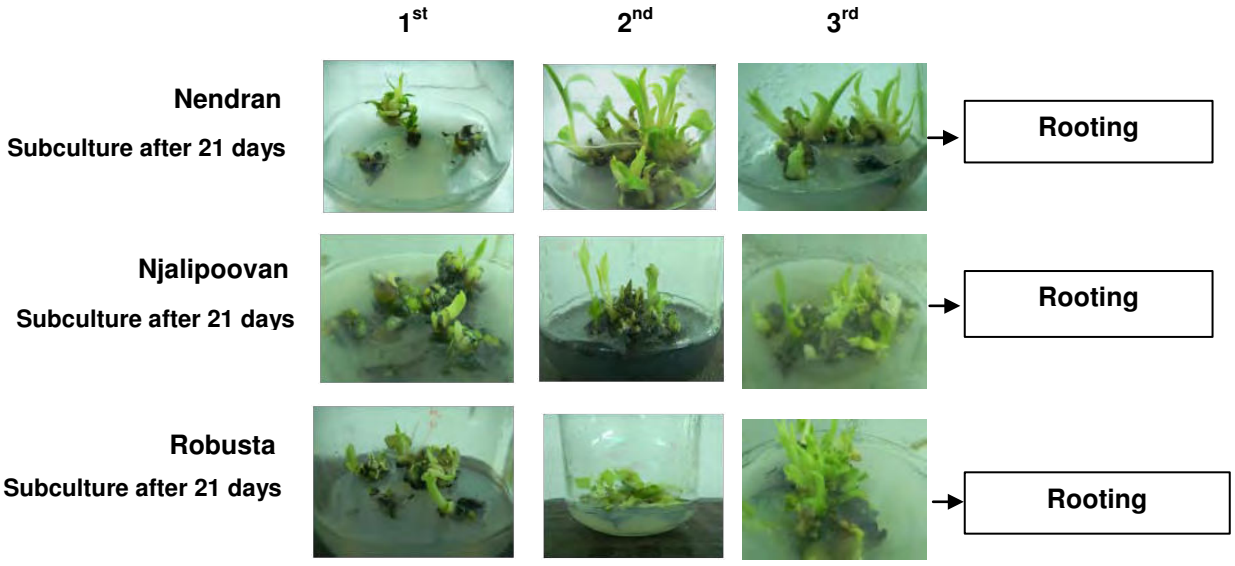

Fig. 40. Subculture of banana inflorescence

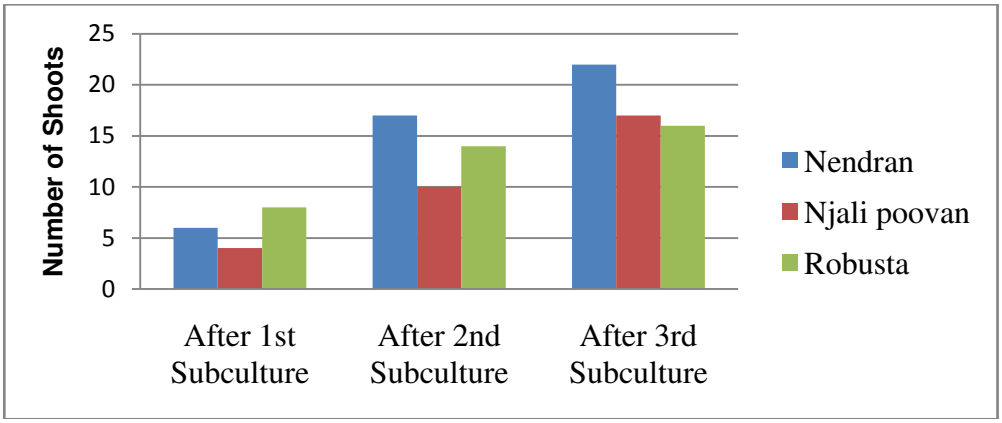

Fig. 41. Shoot production in sequential subculture of banana inflorescence

Stage 3. Rooting

3.1 Rooting of Banana Shoot tip

Objective

To standardize inoculation medium for rooting of banana shoot tip (sucker) s.

Technical Programme

Explants with enough leaves and shoots were separated carefully and transferred to rooting medium and incubated at 25±2°C.

Result

HMS +2mg/l NAA+ 0.5g activated charcoal medium was found effective for red banana and Nendran. HMS +3mg/l IBA+0.5mg/l BA medium responded well for robesta and poovan. HMS medium commonly showed an average performance.

Table 38. Periodical production of roots in different rooting media (Shoot tip)

Banana variety	Medium	Number of roots after nth day				Response
		7	14	21	45	
Red banana	(I) HMS+3mg/l NAA+1mg/l IBA+1g activated charcoal	0.50	2.50	5.00	8.00	+
	(II) HMS +2mg/l NAA+ 0.5g activated charcoal	2.50	4.50	7.50	11.00	+ + +
	(III) HMS +3mg/l NAA+1mg/l IBA	0.50	2.50	5.00	8.00	+
	(IV) HMS +1mg/l NAA+3mg/l IBA	1.50	2.00	4.50	7.00	-
	(V) HMS +1mg/l IAA+3mg/l IBA	1.00	3.00	5.50	9.00	+ +
	(VI) HMS+3mg/l IBA+ 0.5mg/l BA	2.00	3.50	6.50	8.50	+
	(VII) HMS	1.50	3.00	4.00	9.00	+ +
Nendran	(II) HMS +2mg/l NAA+ 0.5g activated charcoal	3.00	5.00	7.00	11.50	+ + +
	(VII) HMS	2.50	4.50	8.50	10.00	+ +
Robesta	(VI) HMS +3mg/l IBA+0.5mg/l BA	2.00	3.50	6.50	8.50	+ + +
	(VII)HMS	2.00	3.00	5.50	8.00	+ +
Poovan	(VI) HMS +3mg/l IBA+0.5mg/l BA	2.50	3.50	6.50	8.50	+ + +
	(VII) HMS	2.00	3.50	5.00	8.00	+ +

Minimum response (+); Medium response (+ +); Maximum response (+ + +)

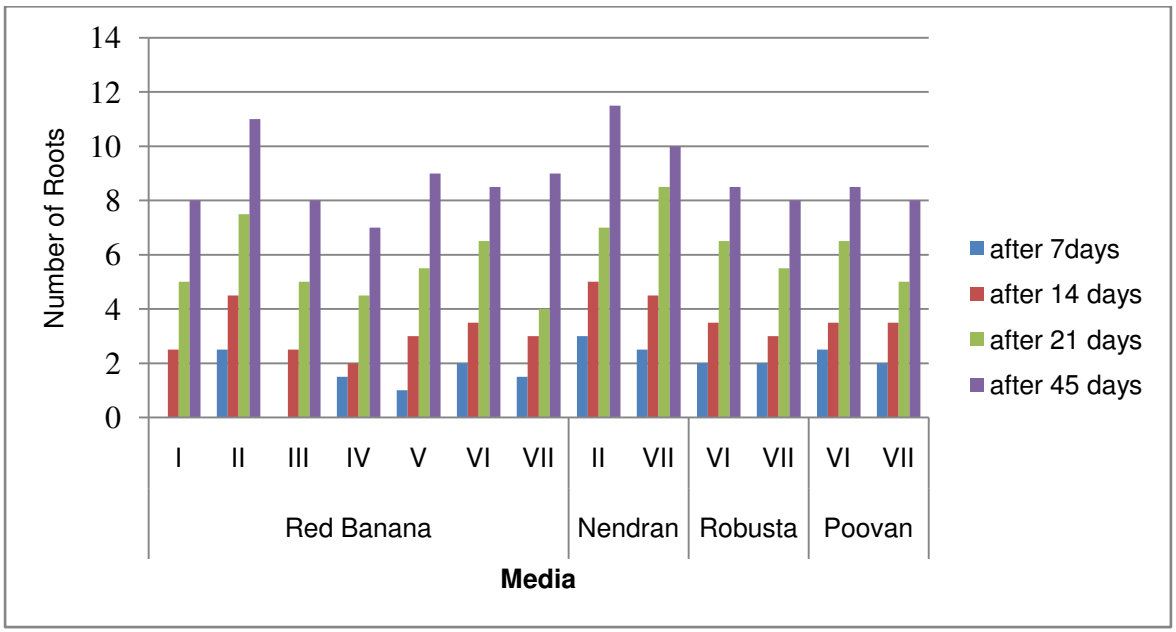

Fig. 42 . Periodical production of banana roots in different rooting media

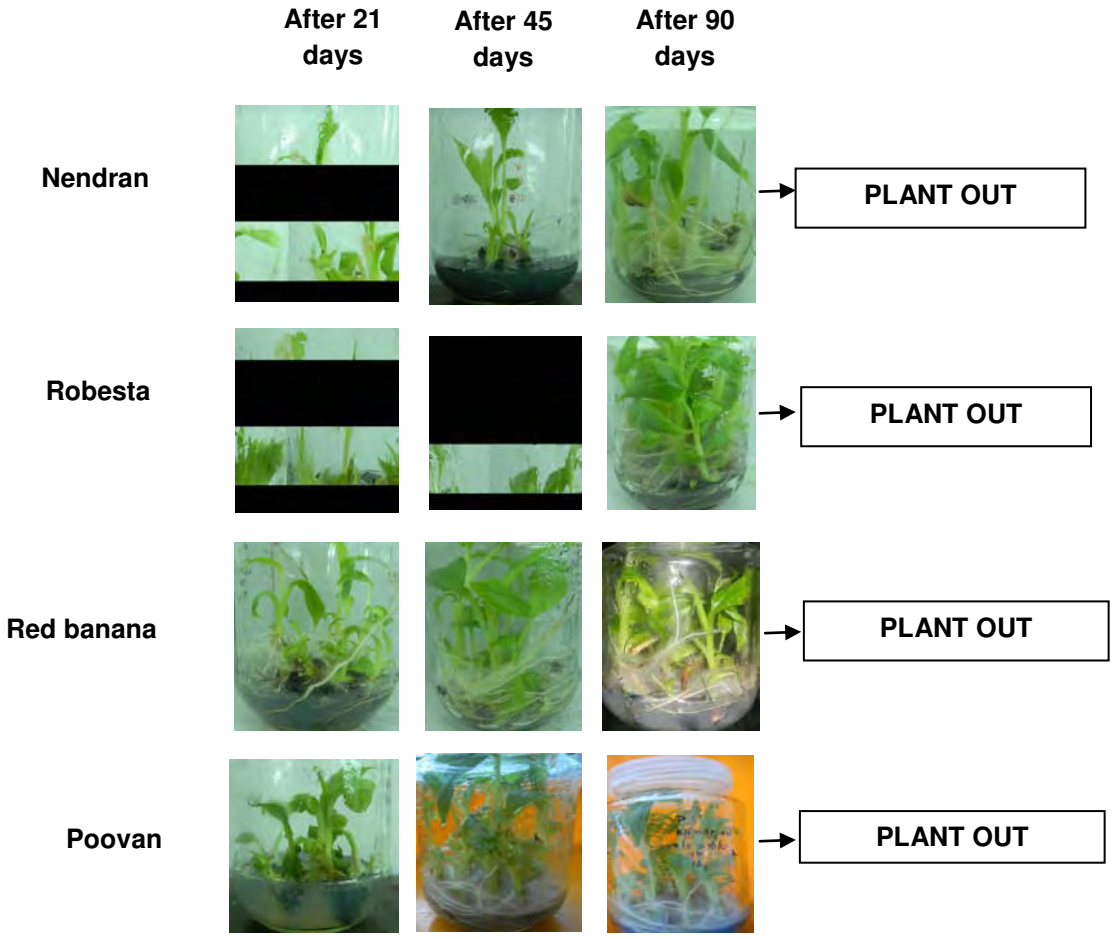

Fig. 43. Rooting of Banana shoot tip (sucker)

3.2 Rooting of Banana Inflorescence

Objective

To inoculate banana Inflorescence to rooting medium

Technical Programme

Explants with enough leaves and shoots were separated carefully, transferred to rooting medium and incubated at 25±2°C.

Result

Nendran rooting was found effective in HMS +2mg/l NAA+ 0.5g activated charcoal (II) medium. HMS+3mg/l NAA+ 1mg/l IBA+1mg/l IAA+ 1g/l activated charcoal(I) and HMS(VII) media showed medium response. HMS +3mg/l NAA+1mg/l IBA(III), HMS +1mg/l NAA+3mg/l IBA(IV), HMS +1mg/l IAA+3mg/l IBA(V) and HMS+3mg/l IBA+ 0.5mg/l BA(VI) showed only a minimum response.

Table 39. Periodical production of roots in different rooting media (Inflorescence)

Medium	Number of roots in n^{th} day				Response
	7	14	21	45	
(I) HMS+3mg/l NAA+ 1mg/l IBA+1mg/l IAA+ 1g/l activated charcoal	2.5	6	7.5	10	+ +
(II) HMS +2mg/l NAA+ 0.5g/l activated charcoal	4.5	8.5	10.5	13.5	+ + +
(III) HMS +3mg/l NAA+1mg/l IBA	0	1.5	3	6	+
(IV) HMS +1mg/l NAA+3mg/l IBA	0	1.5	5	7.5	+
(V) HMS +1mg/l IAA+3mg/l IBA	2	3.5	7.5	9	+
(VI) HMS +3mg/l IBA+0.5mg/l BA	1	1.5	3	5	+
(VII) HMS	3	6.5	8.5	11.5	+ +

Minimum response (+); Medium response (+ +); Maximum response (+ + +)

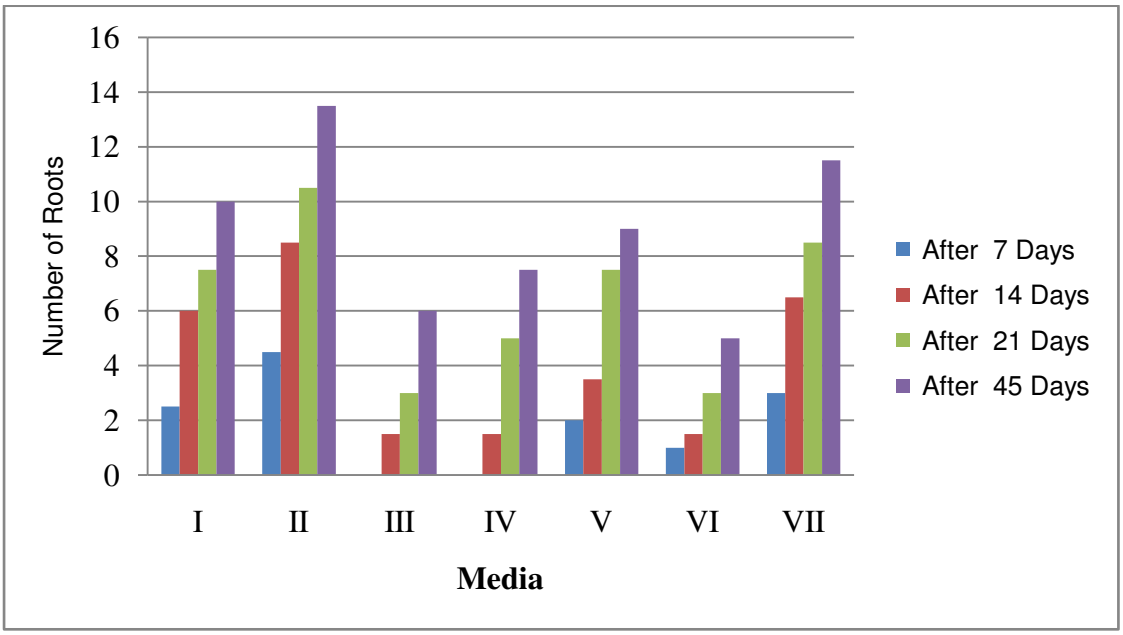

Fig. 44. Periodical production of roots in different rooting media (Inflorescence)

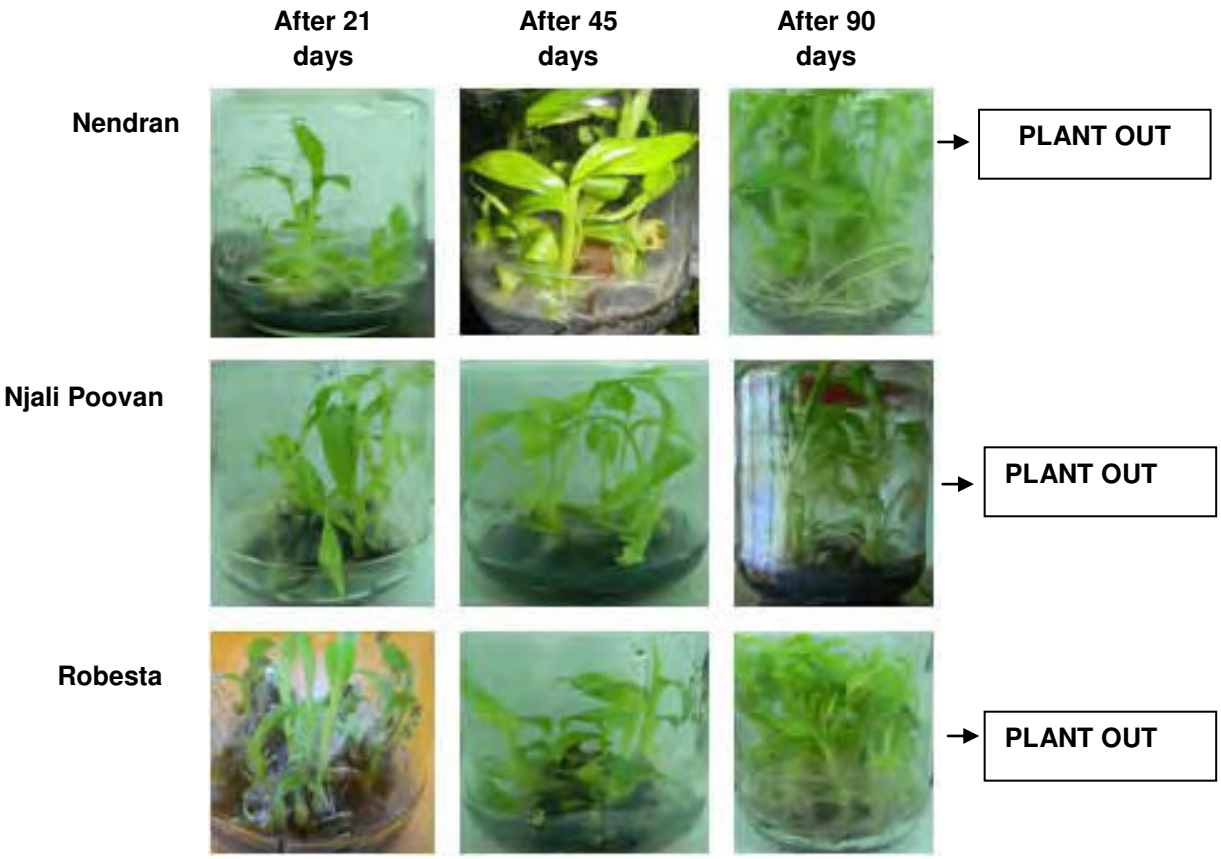

Fig. 45. Rooting of Banana Inflorescence

Stage 4. Hardening of Banana

Fully rooted plants *in vitro* were selected for plant out. Plants were first grown in mist chamber for acclimatizing with climate outside lab. After 2 to 3 week's time they were moved to green house to get adjusted with field conditions. Healthy plants were treated with pseudomonas 2g/l for 30 minutes and planted. Fungal contaminated plants were treated with 2g/l SAAF for 30 minutes and planted. Plants were planted in potting mixture.

Potting mixture was made by mixing 100Kg solarised Soil+10 Kg Cowdung+1kgNeem Cake. The mixture was thoroughly mixed and irrigated well. Potting mixture was kept covered for 4-5 days and Trichoderma (1-2 Kg) was added. It was again mixed and irrigated well. Mixture was kept covered. For one week it was irrigated and mixed at 2 days interval. This mixture was further used for planting.

Micropropagation of Grandnaine

Grandnaine cultures were obtained from Banana Research Station, Kannara. They were sub cultured to BA_2 medium. After the 5^{th} subculture they were planted out.

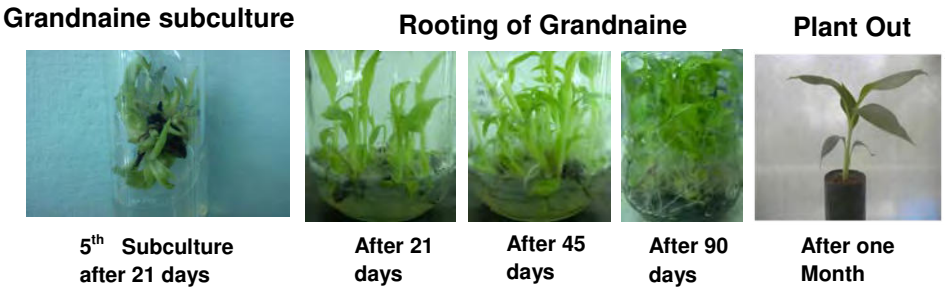

Fig. 46. Micropropogation of Grandnaine

Fig. 47. Banana Inflorescences after one month

4. PLANTING MATERIAL PRODUCTION

Table 40. Planting material production, receipt, target, etc for 2011-12

Crop/Variety	Target (No.)	Production (No.)	Price (Rs.)	Sale (No.)	Receipt (Rs.)	Stock balance (No.)	Target for 2012-13 (No.)
Pineapple TC	5000	1546	10	546	5460	1000	2000
Passion fruit seedlings	1000	1920	5	1820	9100	100	2000
Passion fruit TC & Rooted Cuttings	500	14	10	9	90	5	500
Banana TC	2000	555	15	155	2325	400	2000
Total	8500	4035		2530	16975	1505	6500

5. EXTENSION

5.1 PUBLICATIONS

As per the instruction of the Director of Research during his inspection to the station on 12-1-2012, leaflets on Pineapple Research Station both in English and Malayalam, pineapple and passion fruit were prepared for distribution. General awareness leaflets on pineapple and passion fruit were prepared in Malayalam, got printed and made ready for distribution to the public.

a b c d

Fig. 48 . Leaflets published. a. Pineapple Research Station in malayalam, b. pineapple in Malayalam, c. passion fruit in malayalam, d. Pineapple Research Station in English

5.2 Training Programmes Organized

The following training programmes were conducted during the year.

Table 41. Training programmes conducted during the year

Topic	No. of Trainees	Venue	Date	Name of Scientist
Processing & Value Addition of Fruits	50	Anchalpetty	16/05/11	Ms. Sruthy
Processing & Value Addition of Fruits	50	Pampakuda	17/05/11	Ms. Sruthy
Good Agriculture Practices	50	Muvattupuzha	04/07/11	Dr. PP Joy
Good Agriculture Practices	50	Muvattupuzha	05/07/11	Dr. PP Joy
Processing & Value Addition of Pineapple	50	Kolenchery	27/07/11	Ms. Sruthy

5.3 Radio Talks/ TV Programmes/Audio-Video Cassettes

Table 42. Radio Talks delivered during the year

Topic	Station	Date	Name of Scientist
Pineapple Cultivation, 20min discussion	AIR, Thrissur	27/06/11	Dr. P. P. Joy
Passion Fruit New Varieties	AIR, Thrissur	24/01/12	Dr. P. P. Joy

5.4 VISITORS

Director of Research inspecting the station & discussing with Head of the station on various issues of research & development

Director of Research inspecting basic records & field books of various research projects

Director of Research inspecting TC lab

Director of Research inspecting roof top nursery

Associate Director of Research, RARS Pattambi inspecting the station and reviewing the R & D activities

ADR, RARS pattambi inspecting the basic records, field books & project files of various R & D projects of the centre

Fig. 49. Visitors to the station during the year

SHM team inspecting the station & discussing on SHM projects under taken

Dr. Baby Latha, Prof. & Head, PRC, Vellanikkara , discussing on various aspects of pineapple research & development

Representatives of Agribusiness company visiting the station

Vector Control Lab team and President of pineapple farmers association discussing on mosquito breeding issues in pineapple

A team of pineapple farmers led by Assistant Director of Agriculture Tamil Nadu visiting the station

KAU Scientific team investigating pineapple disease problems in PRS research field

Fig. 49. Visitors to the station during the year (continued)

Table 43. Visitors to the station during the year

Date	Visitors
28/04/11	V. D. Sambji, Direct Board Member (NAPC) K. N. Vijayan Nanbdhy, Retd. DDA (NAPC)
03/05/11	Sree Kumar M. S. , CEO, Foundation for Organic Agriculture & Rural Development, Thottumugham P.O, Aluva, ceo@organicfoundation.in Sreekanth, DDA, Foundation for Organic Agiculture & Rural Development, Thottumugham P.O Atheep, Sub Editor, Haritha bhoomi
18/05/11	Sri. George Joseph, Surya T V
19/05/11	Mr. Renji Thomas, Executive Sales, Rallis India Ltd.
27/05/11	Mr. Shine Damodaran, K. P. S. S. M Dapoli MIDC, Maharashtra
28/05/11	Dr. Om Prakash, Chief Consultant, NHM Sri. Sanjeev S.J. Field Coordinator, SHM
15/06/11	K. Murali Krishna, Executive Director, Clean Foods Ltd. # 845, 5th cross, 10th Main, Indira Nagar 2nd stage, Bangalore murali@cleanfoodsindia.com Atul Sasane, General Manager (Buisiness Development & Investment Officer), ITOCHU India Pvt. Ltd. , Trade Star building, 'c' wing, 6th floor, Andheri-Kurla Road, J.B. Nagar, Andheri Mumbai, atul@itochu.co.in Kafuma Nakano, Group President food Department, ITOCHU India Pvt. Ltd. , Trade Star building, 'c' wing, 6th floor, Andheri-Kurla Road, J.B. Nagar, Andheri, nakano@itochu.co.in Jaime. V. Villacorta, Director, Regional Agricultural Development, Dole Packaged Foods Asia, 9504 Polo molok, South Cotabato, Philippines, Jaime.villacorta@dole.com Mr. N. V. Padnigar, General Manager, Godaveri Bio refineries Ltd. Bangalore Mr. N. V. Kotak, senior Offficer, Girivanvow Dragosi Mandal corporate office, Somaiya Bhavan, M. G. Road, Mumbai Mr. L.S. Pcdliyi, Sr. Manager (Ferms), Godaveri Biorefineries Ltd. , Someerwadi, Ta mudhol Dist. , Begallota, Karnataka
16/07/11	Executives from Godaveri Biorefineries Ltd.
17/07/11	Mr. V. R. Gamsan, Sr. Manager (LARho), Cochin Internatonal Ltd.Mr. Bilu Varghese, Manager (Cargo), CIAL, Airport P. O. , Kochi
18/07/11	Dr. B. Chandra Moli, Advisor, Rallis India Ltd.Mr. John, Rtd. Deputy Director (Agriculture) Mr. Tomy Maximin, Area Sales Manager, Cochin Renji Thomas, Executive Sales
21/07/11	Executives from M/s Gem Agro, M/s Jayavinayaga Agro, M/s Jayakrishna Pesticides (p) Ltd. , No. 59, Kunnji Colony Gandhi Road, Salem,jayakrishnapest@yahoo.co.in
22/07/11	Dr. I. Johnkutty, ADR, RARS, Pattambi
28/07/11	Mr. jose, Deputy Commissioner, Rubber Board, Regional Office , Assam
01/08/11	SHM Team visited
14/09/11	Prof. Meagle joseph, Dept. of Processing, College of Horticulture
27/09/11	V. P. Surendran, Marketing Officer, Margo Biocontrols Pvt. Ltd. , surendranvp2004@yahoo.co.in Biju Basil, K. J. Joseph & Company, Muvattupuzha

01/10/11	K. Nagbhushan Naidu, Field Fresh Foods Pvt. Ltd. , 155/1,155/2,Villa Kalukondapally, Taluk-denkanikore, Krishnagiri Dist, Tamil Nadu Nagbhushan.naidu@fieldfreshfoods.in
28/10/11	Dr. M. C. George, Adv. Ex. member.PSE, INFAM National trustee, Ayavana, Muvattupuzha
19/10/11	Dr. A.K. Babylatha, Professor, PRC, Vellanikara
03/11/11	Josmi N Jose, Honey Baby, Santhigiri college, Vazhithala
31/12/11	Dr. K. R. Viswambharan IAS, Vice chancellor, KAU
05/01/12	Baby John, President Pineapple Farmers Association Dr. N. Pradeep Kumar, officer in charge, VCRC Field Station, Kottayam
12/01/12	Dr. T. R. Gopalakrishnan, Director Of Research, KAU
25/02/12	Dr. K. R. Viswambharan IAS, Vice Chancellor, KAU
21/03/12	Sri. Sathish, AGM, Bangalore, Bayer Crop Science Sri. Srinivasa Rao, Regional Manager, Bayer Crop Science Sri. M. Murugadoss, Field Marketing Manager, Bayer Crop Science

Appendix- 1. Reagents prepared for ELISA

1. Phosphate buffered saline (PBS). pH 7.4 (1litre)

chemicals	1x(g)	10x(g)
NaCl	8	80
KH2PO4	0.2	2
KCl	0.2	2
Na2HPO4	1.16	11.6

2. Coating buffer. pH9.2(1litre)

chemicals	1x(g)	10x(g)
Na2CO3	1.59	15.9
NaHCO3	2.93	29.3
NaNO3	0.28	2.8

3. Substrate buffer. pH 9.8 (100ml)
   9.7ml of diethanolamine is made up to 100ml with distilled water.
4. Substrate solution
   1mg para nitro phenyl phosphate(pnpp) per ml of substrate buffer
5. Wash Buffer (PBS-T)
   Add 0.5ml Tween to 1 l of 1x Phosphate buffered saline.
6. Blocking buffer
   Add 5 g Skin dried Milk to 10ml PBS-T
7. Antibody diluent buffer/ Enzyme conjugate diluent buffer(PBS-TP_O)
   Add 20g poly vinyl pyrrolidone (PVP) and 2g ovalbumin to 1 l PBS-T.
8. Primary Antibody (Ab) concentration rate
   1µl primary Ab: 500µl PBS-TP_O
9. Secondary Antibody concentration rate
   1µl secondary Ab: 10,000µl PBS-TP_O
10. Stop solution
    3M NaOH, ie, 6g NaOH in 50ml distilled water

	Appendix 2. Bill var expenditure details of Pineapple Research Station, Vazhakulam for 2011-12																
	321 Pineapple Research Station																
	321-31-3370 "Research on pineapple"																
Sl.No.	**BRNo**	**110**	**130**	**300**	**142**	**210**	**222**	**226**	**236**	**237**	**330**	**410**	**418**	**821**	**420**	**921**	**Total**
1	1/11-12		19686														**19686**
2	2/11-12					3000											**3000**
3	3/11-12	82017															**82017**
4	4/11-12								3170								**3170**
5	5/11-12					2805											**2805**
6	6/11-12													661			**661**
7	7/11-12	95569															**95569**
8	8/11-12		24518														**24518**
9	10/11-12				3630												**3630**
10	11/11-12				19680												**19680**
11	13/11-12								5404								**5404**
12	16/11-12					905											**905**
13	17/11-12						792										**792**
14	18/11-12										400						**400**
15	19/11-12													661			**661**
16	20/11-12			120													**120**
17	21/11-12					2816											**2816**
18	22/11-12	85405															**85405**
19	23/11-12		20894														**20894**
20	24/11-12				9760												**9760**
21	27/11-12				4070												**4070**
22	30/11-12											6950					**6950**
23	31/11-12								7136								**7136**
24	33/11-12		5824														**5824**
25	34/11-12		19493														**19493**
26	35/11-12				3740												**3740**
27	36/11-12													720			**720**
28	37/11-12									500							**500**
29	39/11-12											2550					**2550**
30	40/11-12										490						**490**
31	41/11-12						415										**415**
32	42/11-12					269											**269**
33	43/11-12	85405															**85405**
34	44/11-12		19493														**19493**
35	45/11-12				4620												**4620**
36	46/11-12				11155												**11155**
37	51/11-12								5366								**5366**
38	53/11-12												990				**990**
39	54/11-12		23611														**23611**
40	55/11-12											2736					**2736**
41	56/11-12			330													**330**
42	57/11-12			420													**420**
43	58/11-12			555													**555**
44	59/11-12			685													**685**
45	60/11-12													664			**664**
47	62/11-12										190						**190**
48	63/11-12						222										**222**
49	64/11-12										800						**800**
50	66/11-12	85405															**85405**
51	67/11-12		31673														**31673**
52	68/11-12				4400												**4400**
53	72/11-12				3700												**3700**
54	76/11-12								3959								**3959**
55	77/11-12					3000											**3000**
56	79/11-12													661			**661**
57	82/11-12		19493														**19493**
58	83/11-12	85405															**85405**
59	84/11-12											2250					**2250**
60	85/11-12				5720												**5720**
61	88/11-12				7910												**7910**
62	91/11-12	1750															**1750**
63	92/11-12		10250														**10250**
64	94/11-12								3903								**3903**
65	99/11-12		33474														**33474**
66	100/11-12													661			**661**
67	103/11-122		20709														**20709**
68	104/11-12				4840												**4840**
69	107/11-12				7000												**7000**
70	111/11-12	85405															**85405**
71	112/11-12								5654								**5654**

Sl.No.	BRNo	110	130	300	142	210	222	226	236	237	330	410	418	821	420	921	Total
72	113/11-12					394											**394**
73	114/11-12						797										**797**
74	116/11-12							6000									**6000**
75	117/11-12			945													**945**
76	118/11-12			150													**150**
77	119/11-12			385													**385**
78	120/11-12			825													**825**
79	121/11-12													994			**994**
80	122/11-12		20709														**20709**
81	123/11-12				5720												**5720**
82	126/11-12				5120												**5120**
83	128/11-12	96073															**96073**
84	129/11-12					3186											**3186**
85	131/11-12											1950					**1950**
86	132/11-12								5564								**5564**
87	133/11-12		6016														**6016**
88	136/11-12													661			**661**
89	137/11-12						2222										**2222**
90	143/11-12		26614														**26614**
91	144/11-12				5170												**5170**
92	147/11-12				5810												**5810**
93	149/11-12	114727															**114727**
94	150/11-12	92143															**92143**
95	153/11-12								5671								**5671**
96	160/11-12									500							**500**
97	163/11-12		21970														**21970**
98	165/11-12	92143															**92143**
99	166/11-12										1393						**1393**
100	169/11-12													711			**711**
101	172/11-12				5940												**5940**
102	176/11-12				8500												**8500**
103	182/11-12								6109								**6109**
104	183/11-12						696										**696**
105	189/11-12											3460					**3460**
106	192/11-12	1172781															**1172781**
107	193/11-12													719			**719**
108	19611-12		21970														**21970**
109	197/11-12				5720												**5720**
110	200/11-12				11000												**11000**
111	203/11-12	92143															**92143**
112	213/11-12								4814								**4814**
113	218/11-12						2369										**2369**
114	219/11-12					5000											**5000**
115	220/11-12													667			**667**
116	222/11-12														14500		**14500**
117	229/11-12	92143															**92143**
118	230/11-12		22494														**22494**
119	236/11-12					2921											**2921**
120	239/11-12								7550								**7550**
121	240/11-12			250													**250**
122	241/11-12			250													**250**
123	242/11-12			250													**250**
124	243/11-12			250													**250**
125	248/11-12															1245	**1245**
126	249/11-12					5000											**5000**
127	255/11-12					9952											**9952**
128	256/11-12					9791											**9791**
129	257/11-12					9902											**9902**
130	258/11-12					7147											**7147**
131	259/11-12													661			**661**
132	265/11-12						2482										**2482**
133	293/11-12					0											**0**
Project Total		**2358514**	**368891**	**5415**	**143205**	**66088**	**9995**	**6000**	**64300**	**1000**	**3273**	**19896**	**990**	**8441**	**14500**	**1245**	**3071753**
	321-31-4449 'Breeding for yield and quality of pineapple'																
1	15/11-12					1039											**1039**
2	25/11-12				8818												**8818**
3	28/11-12					3000											**3000**
4	48/11-12				7400												**7400**
5	61/11-12					217											**217**
6	65/11-12					3000											**3000**
7	71/11-12				11160												**11160**

Sl.No.	BRNo	110	130	300	142	210	222	226	236	237	330	410	418	821	420	921	Total
8	73/11-12					2372											2372
9	78/11-12				3750												3750
10	86/11-12				12820												12820
11	93/11-12										880						880
12	98/11-12					16513											16513
13	105/11-12				11740												11740
14	109/11-12					364											364
15	110/11-12										510						510
16	127/11-12				15100												15100
17	134/11-12					3000											3000
18	135/11-12					2932											2932
19	138/11-12										330						330
20	146/11-12				9890												9890
21	159/11-12										2320						2320
22	174/11-12				11300												11300
23	178/11-12										500						500
24	201/11-12				13005												13005
25	204/11-12					2250											2250
26	217/11-12					345											345
27	233/11-12					5000											5000
28	244/11-12			500													500
29	245/11-12			250													250
30	246/11-12			250													250
31	260/11-12			405													405
32	261/11-12			200													200
33	262/11-12			1005													1005
34	263/11-12			600													600
35	264/11-12			400													400
36	265/-11-12			605													605
37	267/11-12										2257						2257
38	268/11-12			625													625
39	269/11-12			406													406
40	270/11-12			1208													1208
41	271/11-12			250													250
42	272/11-12			3030													3030
43	273/11-12			1250													1250
44	274/11-12			1656													1656
45	275/11-12			406													406
46	276/11-12					10975											10975
47	277/11-12					10975											10975
48	279/11-12					14800											14800
49	280/11-12					4900											4900
50	282/11-12					22625											22625
51	283/11-12					5600											5600
52	284/11-12					6060											6060
53	291/11-12										530						530
54	292/11-12					0											0
	Project Total			**13046**	**104983**	**115967**					**7327**						**241323**
	321-31-3500 'Rsearch in passion fruit '																
1	9/11-12				2970												2970
2	12/11-12				9635												9635
3	26/11-12				7000												7000
4	47/11-12				5315												5315
5	52/11-12					3000											3000
6	70/11-12				6520												6520
7	80/11-12					296											296
8	81/11-12					5000											5000
9	87/11-12				10200												10200
10	102/11-12					7235											7235
11	106/11-12				11140												11140
12	125/11-12				9440												9440
13	139/11-12					191											191
14	148/11-12				8900												8900
15	154/11-12					5000											5000
16	155/11-12				8500												8500
17	173/11-12				10000												10000
18	199/11-12				13425												13425
19	234/11-12					135											135
20	237/11-12					5000											5000
21	278/11-12					10203											10203
	Project Total				**103045**	**36060**											**139105**

Sl.No.	BRNo	110	130	300	142	210	222	226	236	237	330	410	418	821	420	921	Total
	321-31-8841 'Selection of high yeilding superior quality pineapple variety for central zone of Kerala in P.T.D mode '																
1	32/11-12														68200		**68200**
2	49/11-12				7030												**7030**
3	69/11-12				10280												**10280**
4	89/11-12				10900												**10900**
5	96/11-12					11537											**11537**
6	97/11-12				6000												**6000**
7	101/11-12					5000											**5000**
8	108/11-12				13330												**13330**
9	124/11-12				15290												**15290**
10	140/11-12					9701											**9701**
11	141/11-12					16413											**16413**
12	142/11-12				11000												**11000**
13	145/11-12				12500												**12500**
14	156/11-12					6819											**6819**
15	157/11-12					6840											**6840**
16	158/11-12														59900		**59900**
17	161/11-12					193											**193**
18	162/11-12					5000											**5000**
19	164/11-12				3600												**3600**
20	167/11-12				7000												**7000**
21	170/11-12					1980											**1980**
22	171/11-12				8000												**8000**
23	175/11-12				13770												**13770**
24	177/11-12					0											**0**
25	179/11-12					4500											**4500**
26	181/11-12					15146											**15146**
27	184/11-12														79800		**79800**
28	185/11-12														8750		**8750**
29	186/11-12														11000		**11000**
30	187/11-12														6900		**6900**
31	188/11-12				5000												**5000**
32	190/11-12					19000											**19000**
33	191/11-12				3500												**3500**
34	195/11-12					10208											**10208**
35	198/11-12				14560												**14560**
36	202/11-12					11500											**11500**
37	206/11-12					14000											**14000**
38	207/11-12					12500											**12500**
39	208/11-12					10250											**10250**
40	209/11-12					8810											**8810**
41	210/11-12				7500												**7500**
42	214/11-12														4619		**4619**
43	215/11-12					5061											**5061**
44	216/11-12					586											**586**
45	221/11-12				4000												**4000**
46	223/11-12				7200												**7200**
47	224/11-12				6750												**6750**
48	225/11-12				14745												**14745**
49	226/11-12				14505												**14505**
50	227/11-12				14145												**14145**
51	228/11-12					490											**490**
52	235/11-12					173											**173**
53	238/11-12					10000											**10000**
54	247/11-12					4070											**4070**
55	250/11-12					8390											**8390**
56	251/11-12					8390											**8390**
57	252/11-12					2940											**2940**
58	253/11-12				8000												**8000**
59	254/11-12				7950												**7950**
60	281/11-12														199000		**199000**
61	285/11-12				7950												**7950**
62	286/11-12				8100												**8100**
63	287/11-12				11415												**11415**
64	288/11-12				10870												**10870**
65	289/11-12				12695												**12695**
66	290/11-12				11280												**11280**
Project Total					**288865**	**209497**									**438169**		**936531**
Station Total		**2358514**	**368891**	**18461**	**640098**	**427612**	**9995**	**6000**	**64300**	**1000**	**10600**	**19896**	**990**	**8441**	**452669**	**1245**	**4388712**