KERALA AGRICULTURAL UNIVERSITY

PINEAPPLE RESEARCH STATION

Vazhakulam, Muvattupuzha, Ernakulam District, Kerala-686 670

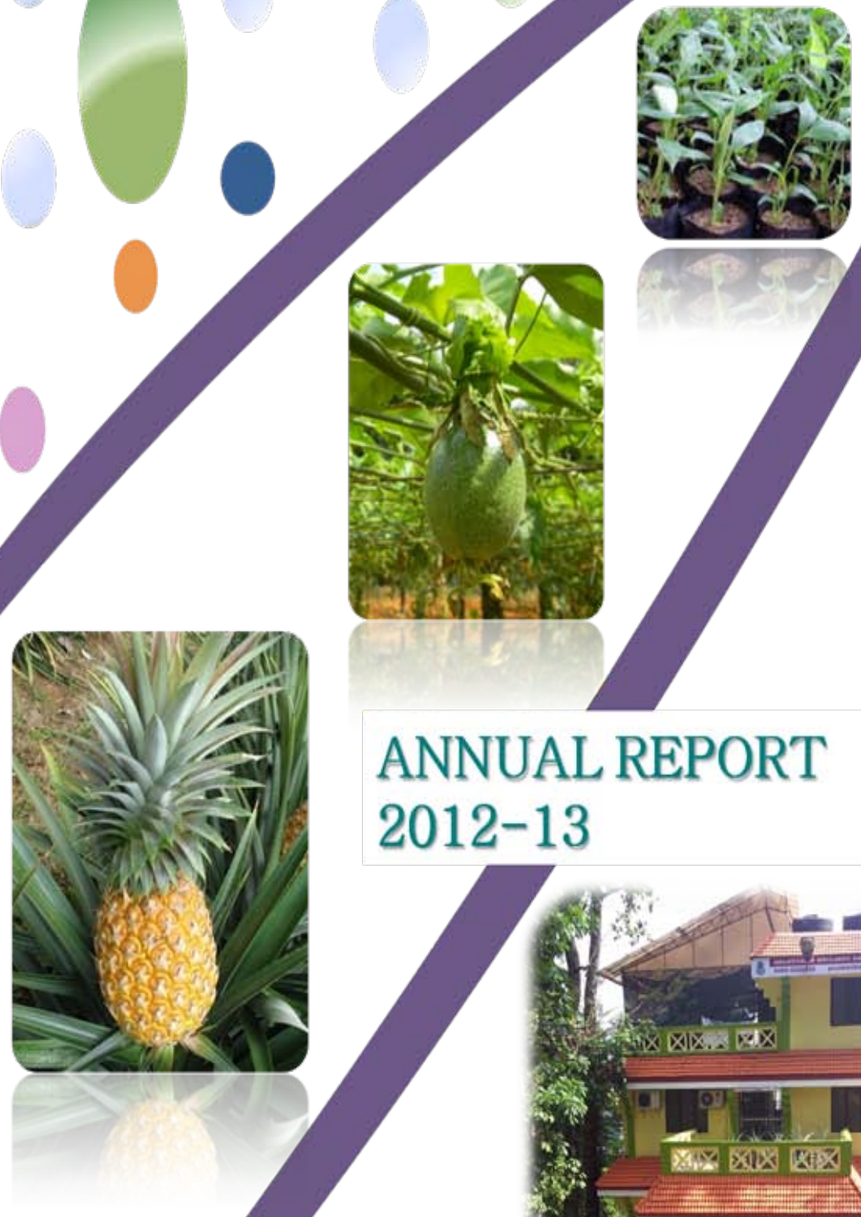
Tel. & Fax: 0485-2260832, Mobile: 9446010905

Email: prsvkm@kau.in, prsvkm@gmail.com

Web: www.kau.edu/prsvkm, http://prsvkm.tripod.com

Dr. P. P. Joy

Research and Development Report 2012-13



ANNUAL REPORT 2012-13

PINEAPPLE RESEARCH STATION
KERALA AGRICULTURAL UNIVERSITY

PINEAPPLE RESEARCH STATION
VAZHAKULAM

Annual Research and Development Report for 2012-13
(01.04.2012 to 31.03.2013)

Dr. P.P. JOY
Associate Professor & Head

Technical Support

Anjana R., Soumya K.K., Sherin C. George,
Prince Jose, Jasna V., Justin T. Jose

KERALA AGRICULTURAL UNIVERSITY
PINEAPPLE RESEARCH STATION
Vazhakulam, Muvattupuzha, Ernakulam District, Kerala, PIN-686 670
Tel. & Fax: 0485-2260832, Mobile: 9446010905
Email: prsvkm@kau.in, prsvkm@gmail.com
Web: www.kau.edu/prsvkm, http://prsvkm.tripod.com

31.03.2013

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.

Protocols for the micropropagation of pineapple and banana have been standardized. Production of tissue culture pineapple is continued. Tissue culture production of banana is augmented. Micro propagation of pineapple such as MD-2, Kew and banana such as Nendran, Red Banana & Poovan were done. Media standardization experiments were carried out in banana. MD-2 and Kew were multiplied in MS+4mg/lBA+1mg/l NAA. MD-2 rooting media was redefined to MS+1mg/IBA+1mg/l NAA media. Planting materials in the form of seedlings, TC plants and rooted cuttings were mass produced and sold out. Diagnostic team visits were conducted. Pest and disease samples of station field, nursery, tissue culture lab and of farmers were studied. Plant Health Releases were done to get suggestions from resource persons.

In the field study 'Selection of high yielding superior quality pineapple variety for central zone of Kerala in PTD mode' 11 pineapple types are being evaluated in RBD with 3 replications. Growth, yield and quality observations recorded are presented. Mauritius and MD-2 varieties are showing good results. In the study 'Breeding for Yield and Quality of Pineapple' to develop pineapple varieties suitable for processing and table purpose through hybridization, the progenies were shortlisted based on fruit weight and brix value and 186 superior plants were selected, replanted and being evaluated. Yield and quality observations recorded are presented.

The 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 sanctioned by Kerala State Council for Science, Technology and Environment to identify a high yielding superior quality passion fruit variety for commercial cultivation in Kerala is in the first year of implementation involving land preparation, experimental layout, pandal preparation, preparation of planting materials, planting and crop management (shading, irrigation, manuring, plant protection, training on pandal, pollination, etc). Growth, yield and quality observations are being recorded.

A Project Proposal under Pineapple Mission entitled 'Development of Pineapple Sector in Kerala in Mission Mode' at a budget of Rs. 137.8 lakh for 3 years was submitted to the Government of Kerala through the Director of Extension, Kerala Agricultural University on 28.07.2012 with the objective of To boost the production and productivity of superior quality GI registered Vazhakulam pineapple in Kerala through comprehensive multi-pronged integrated approach in mission mode.

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.

Student projects are also undertaken at the station. Pest and disease problems of 50 farmers were attended to during last year. 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.

CONTENTS

	Page
A. STATION AT A GLANCE	6
B. ONGOING PROJECTS	9
C. DETAILED RESEARCH REPORT	10
1. RESEARCH ON PINEAPPLE	10
1.1 Micropropagation	10
1.1.1 Micropropagation Of MD-2	10
1.1.1.1 Comparison of callus induction of MD-2 Sucker with MD-2 Crown	10
1.1.1.2 Rooting Efficiency Of MD-2 shoots	13
1.1.2 Micropropagation Of Kew	16
1.2 Selection of High Yielding Superior Quality Pineapple Variety for Central Zone of Kerala in PTD mode	20
1.2.1 Shelf Life Studies of Pineapple Variety MD-2	33
1.3 Breeding for Yield and Quality of Pineapple	35
1.3.1 Evaluation of Shortlisted pineapple hybrid lines	*35*
1.4 Plant Protection Studies	40
1.4.1 Efforts for Maintaining Contamination Free Tissue Culture Lab and Nursery	40
1.4.1.1 Identification of Tissue Culture Contaminations	41
1.4.1.1.1 Identification of fungal contaminations in plant tissue culture laboratory	41
1.4.1.1.2 Identification of bacterial contaminations in plant tissue culture laboratory	45
1.4.1.2 Identification of Contaminations in Nursery	47
1.4.1.2.1 Potting Mixture Analysis of Passion Fruit Roof Top Nursery	47
1.4.1.2.2 Identification of fungal pathogen in soil samples through serial dilution technique	48
1.4.2 Identification of Pathogens from Diseased Plants	51
1.4.2.1 Isolation and identification of pathogen for white leaf spot disease in Pineapple	51
1.4.3 Bacteriological Analysis of Water Sample from Tissue Culture Lab	52
1.4.4 Antifungal Sensitivity Tests	53
1.4.4.1 Response of the fungi *Cercospora spp.* to different fungicides with varying concentrations	53

1.4.5 Effect of Copper sulphate ($CuSO_4.5H_2O$) on *Candida spp.* isolated from the rotted pineapple sample	55
1.4.6 Plant Health Clinic Releases	56
1.4.7 Diagnostic Team visits	58
2. RESEARCH ON PASSION FRUIT	59
2.1 Study on germination and seed viability of passion fruit seedlings	59
2.2 Study on the effect of nodal length on the rooting of purple passion fruit stem cuttings	61
2.3 KSCSTE Project: Evaluation of Passion fruit types for commercial cultivation in Kerala	63
3. RESEARCH ON BANANA	73
3.1 Media Standardization for the Promotion of Multiplication of Banana	*73*
4. MASS PRODUCTION & SALE	76
5. EXTENSION ACTIVITIES	78
5.1 Trainings Attended	78
5.2 Training Programmes Organized	79
5.3 Media Coverage	80
5.4 Publications	80
5.5 New Project Proposal	81
6. VISITORS	82
7. APPENDICES	86
7.1 Diseases of pineapple (*Ananas comosus*): pathogen, symptoms, infection, spread & management	86
7.2 Diseases of passion fruit (*Passiflora edulis*): pathogen, symptoms, infection, spread & management	100
7.3 Insect pests of passion fruit (*Passiflora edulis*): hosts, damage, natural enemies and control	116
7.4 Fruits, benefits, processing, preservation and pineapple recipes	129
7.5 Protocol for micropropagation of pineapple (MD-2)	149
7.6 Protocol for micropropagation of banana	153
7.7 Basic fruit analysis of pineapple: a laboratory manual	159
7.8 Recent trends in biology	167
7.9 Development of pineapple sector in Kerala in mission mode	188
7.10 Bill var expenditure details of Pineapple Research Station, Vazhakulam for 2012-13	198

RESEARCH AND DEVELOPMENT REPORT OF PINEAPPLE RESEARCH STATION, VAZHAKULAM FOR 2012-2013

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 Programme (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

Dr. P. P. Joy, Associate Professor and Head, +919446010905, joyppkau@gmail.com
Sri. Justin T. Jose, Senior Grade Assistant, +919744469876
Ms. Anjana R, Project Fellow (KSCSTE Project on Passion fruit)
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'.

B. ONGOING PROJECTS

Table 1. Ongoing research projects of the station during 2012-13

Head of Account	Project Title	Funding Agency	PRS File No.	DoR File No.	Finance File No.
321-31-3370	Research On Pineapple	KAU Plan (FF/10-00-02-95/VZM(15) KHDP)	PRS/R16/10	R8/66091/04	
321-31-4449	Breeding for yield and quality of pineapple	KAU Plan (FR/09-00-03-2001/VZK(9) KAU)	PRS/R17/10	R8/70507/03	
321-31-8841	Selection of high yielding superior quality pineapple variety for central zone of Kerala in PTD mode	KAU Plan	PRS/R32/10	R8/66824/10	EP/B1/10945/11
321-31-3500	Research In Passion Fruit	KAU Plan	PRS/R29/10	R6/65723/03	
321-31-9027	Evaluation Of Passion Fruit Types For Commercial Cultivation In Kerala	KSCSTE (File No. 013/SRSAGR/2010/CSTE)	PRS/R33/10	R2/60024/12	EP/A1/4077/12

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

1.1 Micropropagation

The mass production of MD-2 and Kew varieties of pineapple were done via micropropagation. The medium for the growth of the cultures contained all the salts and vitamins of Murashige and Skoog medium supplemented with different cytokinins and auxins.

1.1.1 Micropropagation Of MD-2

1.1.1.1 Comparison of callus induction of MD-2 Sucker with MD-2 Crown

Objective

To compare the callus induction in both MD-2 sucker and MD-2 crown

Technical Programme

The two types of explant sources in MD-2 micropropagation (crown & sucker) were undergone multiplication in the same medium for the same time period. They were inoculated in MS+4mg/l BA + 1mg/l NAA medium for 21 days to obtain maximum calli. MD-2 calli initiation was analyzed to identify the most fruitful explant source for MD-2 micropropagation.

Result & Discussion

The cultures were observed for callus % and the growth score determined how many cultures obtained during a particular subculture. Growth score 1 to 4 elucidated the following.4 – *number of cultures 15 or above 15*, 3 - *cultures 10 or between 10 & 15*, 2 - *cultures 5 or between 5 & 10*, 1 - *culture 1 or between 1 & 5*. Callus index was attained by multiplying callus % with growth score.

The 4^{th} and 5^{th} subculture stages of both MD-2 sucker and MD-2 crown were compared and analyzed. MD-2 sucker cultures gave maximum callus index at 4^{th} stage where as MD-2 crown showed a minimum callus index at both 4^{th} and 5^{th} stages of subculture.

MD-2 sucker yielded quicker response in callus induction when compared with MD-2 crown.

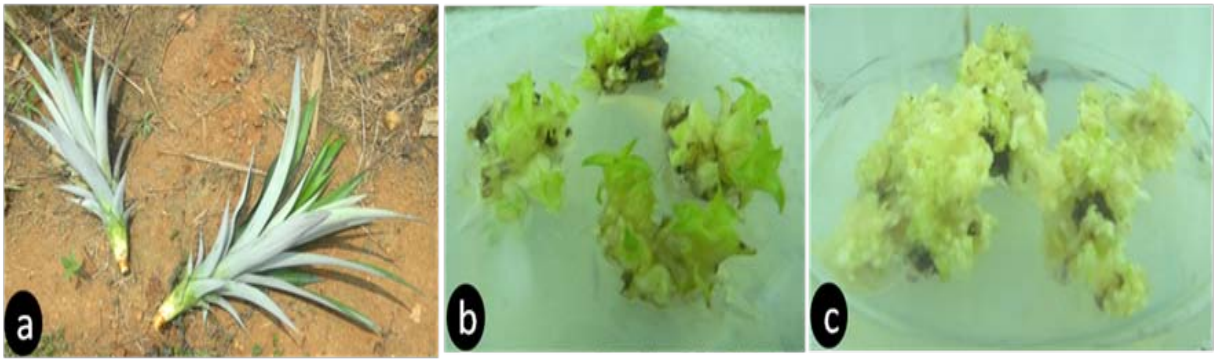


Figure 1. MD-2 Callus initiation via sucker: (a) Sucker (b) 4th subculture after 21 days (c) 5th subculture after 21 days

Table 2. MD-2 sucker explant subculture comparison

4th Subculture			*5th Subculture*		
Callus% (c)	Growth Score (g)	Callus Index (c x g)	Callus% (c)	Growth Score (g)	Callus Index (c x g)
100	4	400	83.3	2	166.6
100	4	400	100	2	200
100	2	200	100	3	300
100	1	100	88.89	2	177.78
100	2	200	100	2	200
100	1	100	100	1	100
100	4	400	100	1	100
100	4	400	100	1	100
			100	4	400
			100	4	400

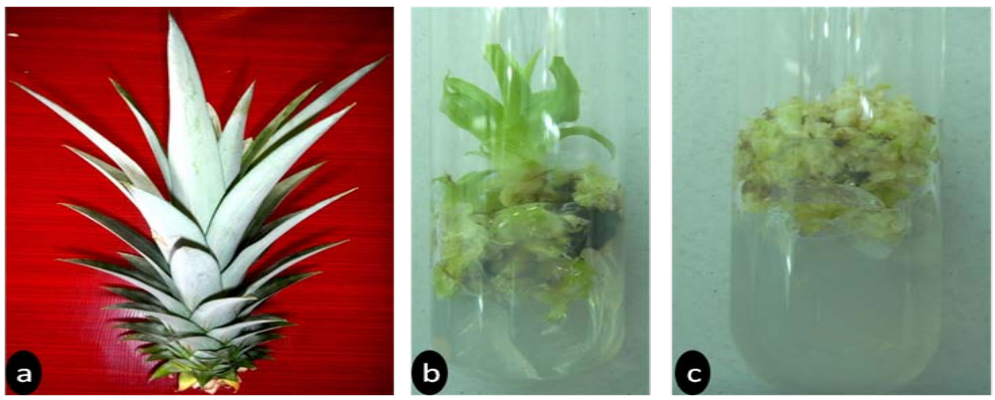


Figure 2. MD-2 Callus initiation via crown: (a) Crown (b) 4th subculture after 21 days (c) 5th subculture after 21 days

Table 3. MD-2 crown explant subculture comparison

4th Subculture			*5th Subculture*		
Callus% (c)	Growth Score (g)	Callus Index (c x g)	Callus% (c)	Growth Score (g)	Callus Index (c x g)
100	4	400	100	1	100
100	4	400	100	4	400
			100	2	200
			100	1	100
			100	1	100
			100	1	100
			100	2	200
			100	1	100
			100	2	200
			100	1	100
			100	1	100
			100	1	100
			100	1	100
			100	1	100
			100	1	100
			100	2	200
			100	2	200
			100	4	400

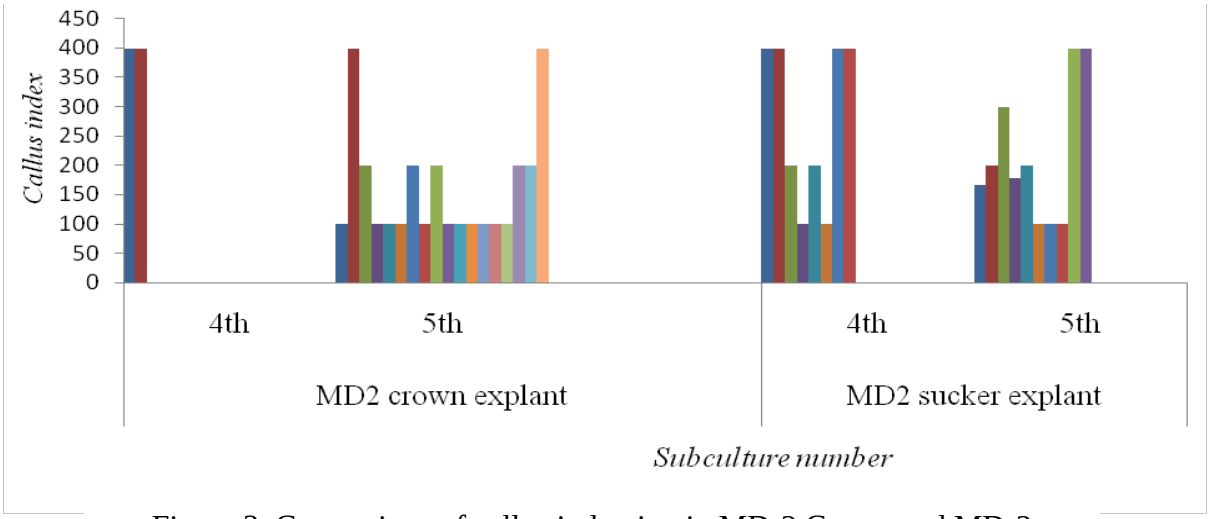


Figure 3. Comparison of callus induction in MD-2 Crown and MD-2

1.1.1.2. Rooting Efficiency of MD-2 shoots

Objective

To study and compare the rooting efficiency of MD-2 shoots in 1mg/l IBA + 1mg/l NAA medium with 1.5 mg/l IBA + 1.5 mg/l NAA medium

Technical Programme

MD 2 shoots of 1 – 2 cm height were carefully separated and inocculated to the aforesaid medium. Each culture bottle contained 10 shoots of similar growth pattern. The cultures were observed periodically for 90 days on each 30th day the number of shoots rooted and the number of roots sprouted were closely observed and tabulated. At the end of 90 days the average number of roots developed per shoot was calculated. Thus getting the rooting percentage.

Results & Discussion

The root sprouting was observed towards the end of 30 days. Further root proliferation occured during the subsequent days. The roots gradually increased its length. Profuse rooting was noticed after 90 days. The response of MD-2 shoots to *a* (1mg/l IBA + 1mg/l NAA) and *b* (1.5 mg/l IBA + 1.5 mg/l NAA) medium was compared. The medium *a* showed the highest rooting % of 11 with a total average of 6.8. The highest rooting % for medium *b* was 9.2 with a total average of 6.44. The medium *a* showed the maximum roots per plant. Our earlier studies proved the medium 1mg/l IBA + 1mg/l NAA best for root proliferation. Further we scrutinized the results including more parameters to analyze the individual shoots' rooting efficiency. Also the cultures were visually assessed for any kind of variations affected. The medium 1.5 mg/l IBA + 1.5 mg/l NAA expressed certain variations in leaf characters like leaf shortening, leaf thickening, leaf colour, spiny leaf and plant height.

In every aspect the medium *a* 1mg/l IBA + 1mg/l NAA resulted in increased root formation with least susceptibility to variation.

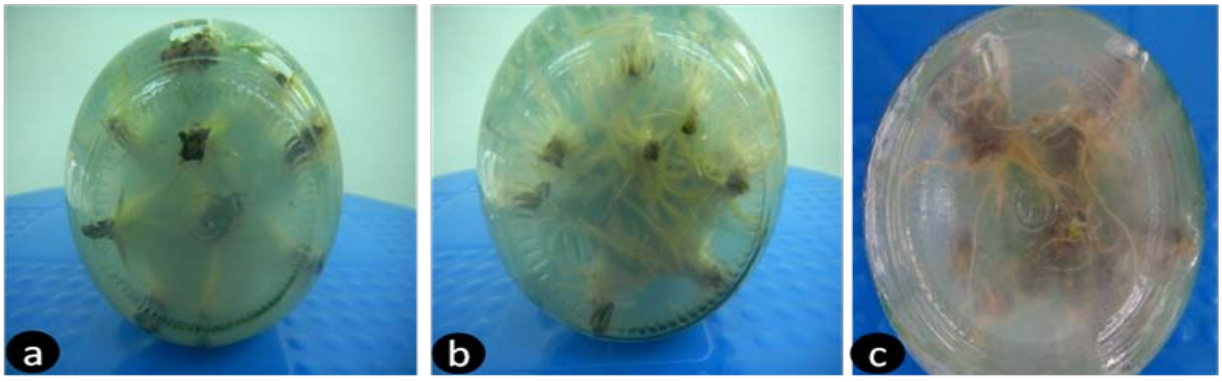


Figure 4. MD-2 rooting: (a) rooting observed after 30 days (b) after 60 days (c) after 90 days

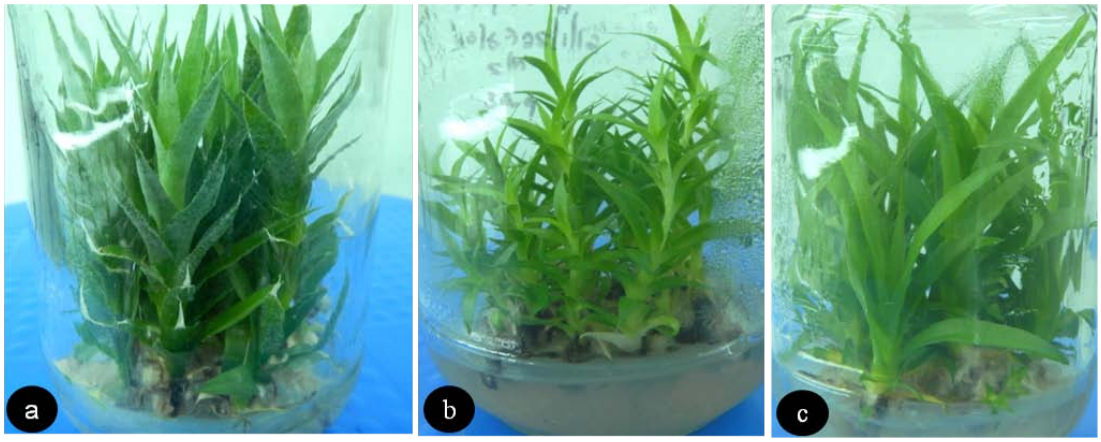


Figure 5. Variations observed in MD-2 rooting: (a) spiny & thickened leaves (b) small leaves (c) normal MD-2 plant

Table 4. Rooting Response of MD-2 Shoots in 1mg/l IBA + 1mg/l NAA medium

Plant name	*After 30 days*		*After 60 days*		*After 90 days*		*Roots per Plant*
	No. of roots	*No. of rooted shoots*	*No. of roots*	*No. of rooted shoots*	*No. of roots*	*No. of rooted shoots*	
M1	25	10	38	10	60	10	6
M2	24	9	36	10	72	10	7.2
M3	42	9	75	10	86	10	8.6
3d	34	10	56	10	66	10	6.6
5b8	16	10	27	10	70	10	7
5b7	7	5	12	6	42	10	4.2
M4	10	8	18	10	32	10	3.2
M5	40	10	72	10	110	10	11
M6	41	10	75	10	62	10	6.2
M7	38	9	53	10	60	10	6
M8	46	8	71	10	86	10	8.6
M9	15	10	23	10	70	10	7
							Mean 6.8

Table 5. Rooting Response of MD-2 Shoots in 1.5 mg/lIBA + 1.5 mg/l NAA medium

Plant name	*After 30 days*		*After 60 days*		*After 90 days*		*Roots per Plant*
	No. of roots	*No. of rooted shoots*	*No. of roots*	*No. of rooted shoots*	*No. of roots*	*No. of rooted shoots*	
M1	20	8	34	10	40	10	4
M2	51	10	52	10	55	10	5.5
M3	17	7	30	8	63	10	6.3
3d	48	10	46	10	62	10	6.2
5b8	50	10	86	10	92	10	9.2
5b7	24	6	38	8	44	10	4.4
M4	66	10	75	10	80	10	8
M5	44	10	54	10	60	10	6
M6	44	10	45	10	49	10	4.9
M7	48	10	74	10	90	10	9
M8	30	8	56	10	74	10	7.4
M9	46	10	50	10	64	10	6.4
							Mean 6.4

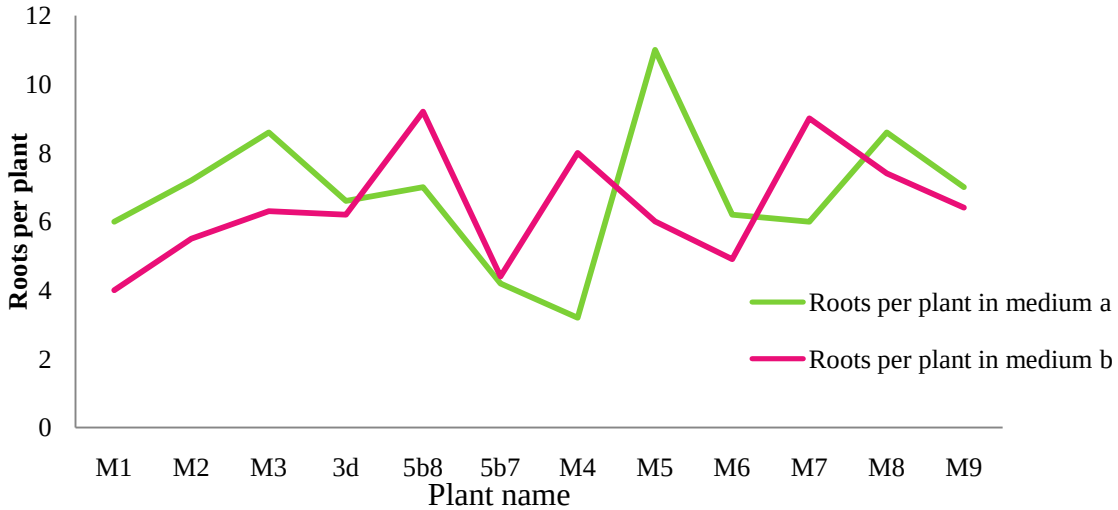


Figure 6. Rooting response of MD2 in *a* (1mg/l IBA +1mg/l NAA) and *b* (1.5mg/l IBA+1.5mg/l NAA) media

1.1.2 Micropropagation of Kew

Objective

To mass produce Kew plants through callus induction

Technical Programme

Stage 1. Fresh Inoculation

The healthy explants (crown) were surface sterilized in various levels. Running tap water wash, soap and fungicide stirring and finally antibiotic dip were the initial level of surface sterilization for pathogen elimination. The treated explants were surface sterilized in 0.1 % $HgCl_2$ for 5min. and rinsed in sterile water three times with continuous shaking. They were inoculated to MS + 3mg/l BA medium. The explant growth progression and bud development were examined for 21 days.

Stage 2. Multiplication

The multiplication in Kew is a two step process- *Bud Proliferation & Callus Induction*

Stage 2. a. Bud Proliferation

The initial bud development was enhanced by inoculating to another medium with more BA strength, MS + 5mg/l BA medium. The green culture or the bud developed cultures were sectioned and inoculated to the medium. The cultures were observed for the next 21 days for increase in bud number from 1- 12.

Stage 2. b. Callus Induction

The buds developed were dissected from the main culture for inoculation to another medium MS + 4mg/l BA + 1mg/l NAA for callus initiation. The cultures thus obtained were analyzed for further 21 days.

Results & Discussions

The fresh inoculated cultures were examined for 21 days in 7 day period. At the end of first seven days the cultures showed creamy to slight green and slight green to green or bud formation. The observations were quantified by visual scoring.

When the cultures were further inoculated to MS + 5mg/l BA medium they gave more buds with improved bud proliferation. The bud number increased from 1 to 12 numbers within 21 days.

Although the cultures were proliferated, mass production of propagules could be achieved only through callus initiation. The cultures in MS + 4mg/l BA + 1mg/l NAA medium showed callus initiation. A profuse growth was not achieved. They gave only a medium growth of calli. Hence improved callus induction along with plant regeneration in Kew is a subject matter under study.

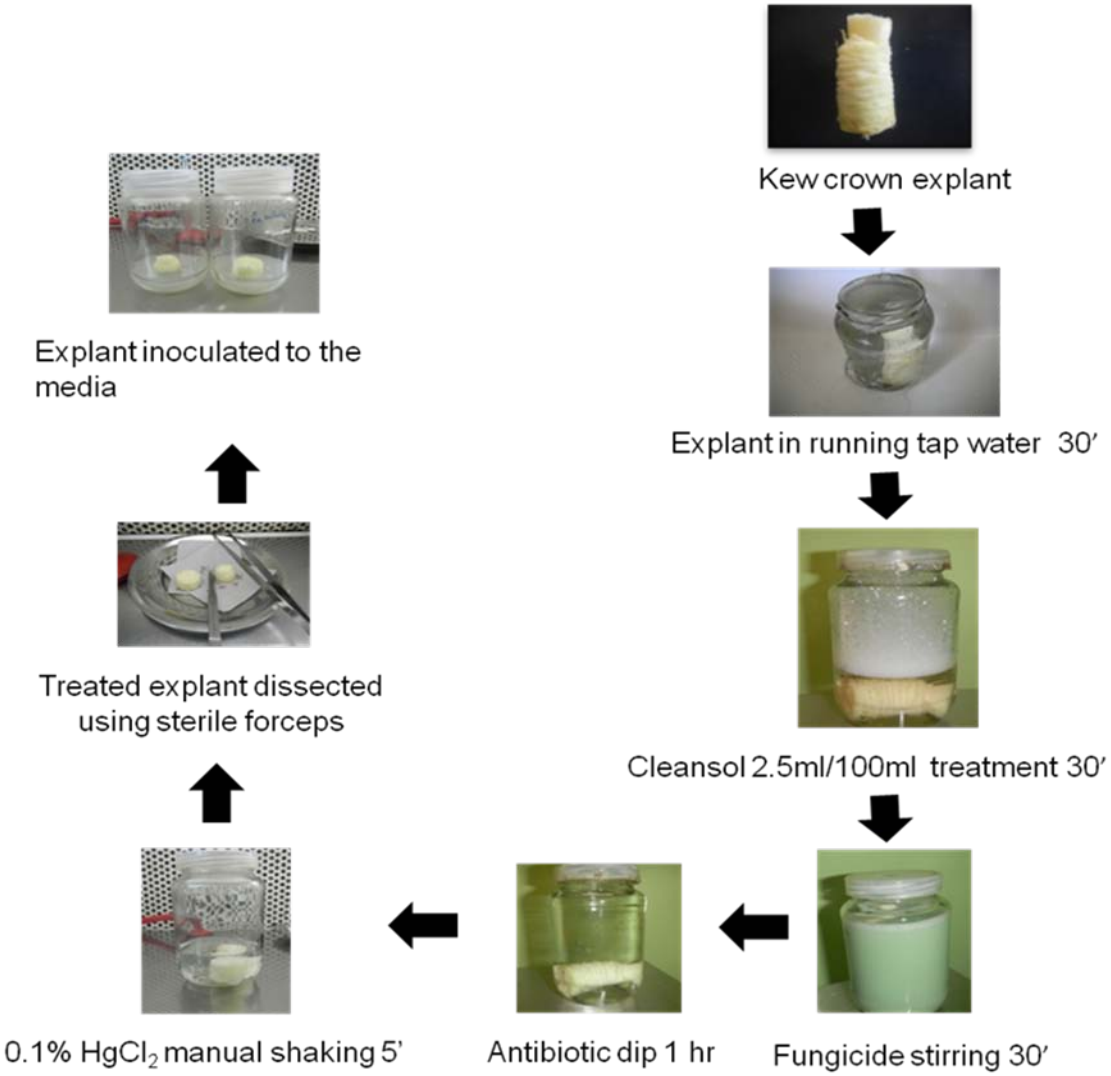


Figure 7. Fresh inoculation protocol for Kew

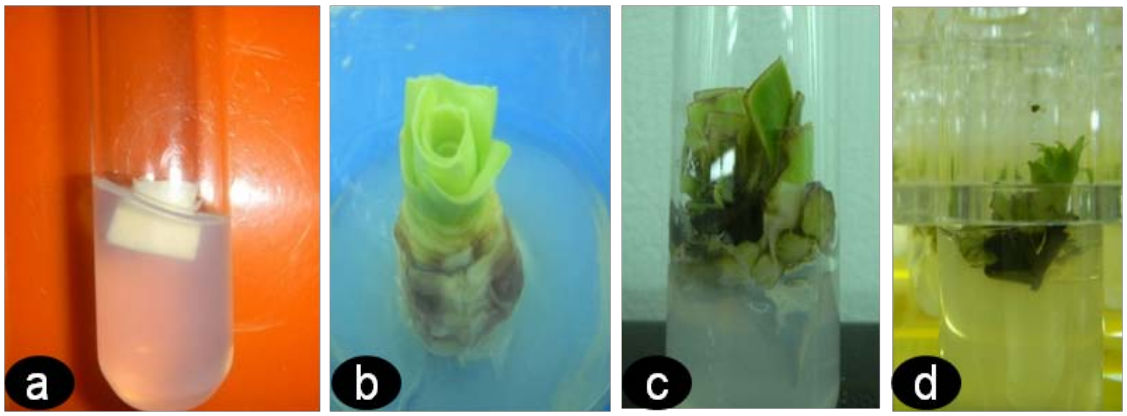


Figure 8. Kew fresh inoculation: (a) 0^{th} day (b) after 7 days (c) after 14 days (d) after 21 days

Table 6. Progressive Response Of Kew Fresh Inoculation

Plant Name	*After 7 Days*	*After 14 Days*	*After 21 Days*	*Visual Scoring*
K1	Slight Green	Green	1 bud	3
K2	Creamy	Slight Green	Green	2
K3	Creamy	Slight Green	Green	2
K4	Slight Green	Green	Green	2
K5	Creamy	Slight Green	Green	2
K6	Creamy	Slight Green	Green	2
K7	No change	creamy	Slight Green	1
K8	Creamy	Slight Green	Green	2

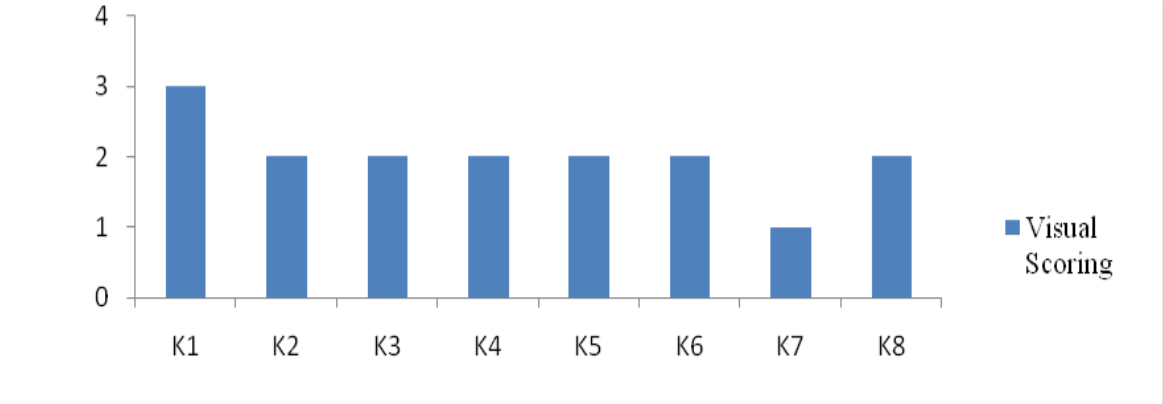


Figure 9. Periodical response of Kew during fresh inoculation

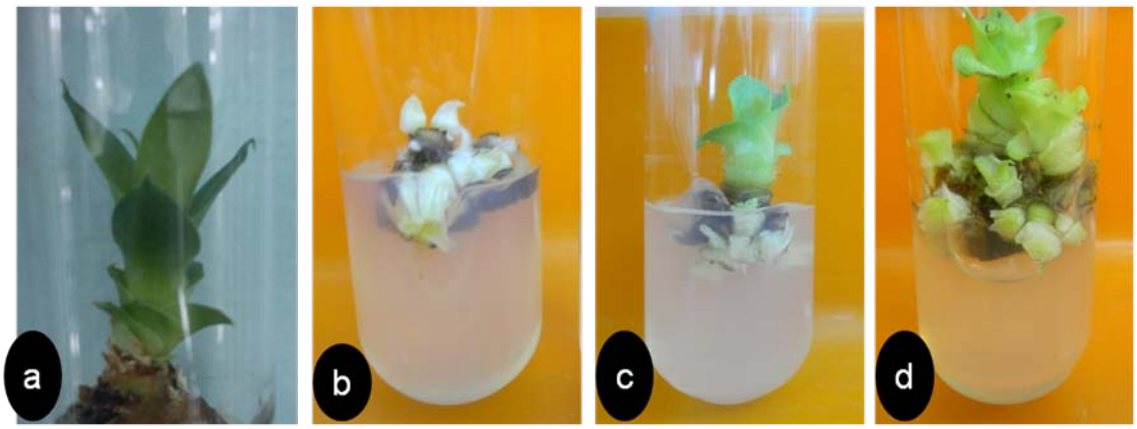


Figure 10. Kew Bud proliferation: (a) 0th day (b) after 7 days (c) after 14 days (d) after 21 days

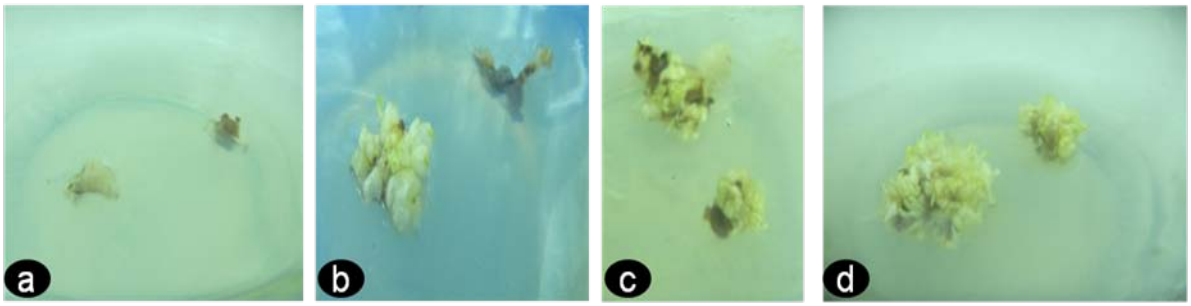


Figure 11. Kew Callus Initiation: (a) 0th day (b) after 7 days (c) after 14 days (d) after 21 days

Table 7. Bud proliferation in Kew

Plant Name	*Bud Number*		
	After 7 Days	*After 14 Days*	*After 21 Days*
K1	2	6	12
K2	1	1	2
K3	1	1	1
K4	1	2	2
K5	2	4	8
K6	1	2	4
K7	0	1	1
K8	1	1	1

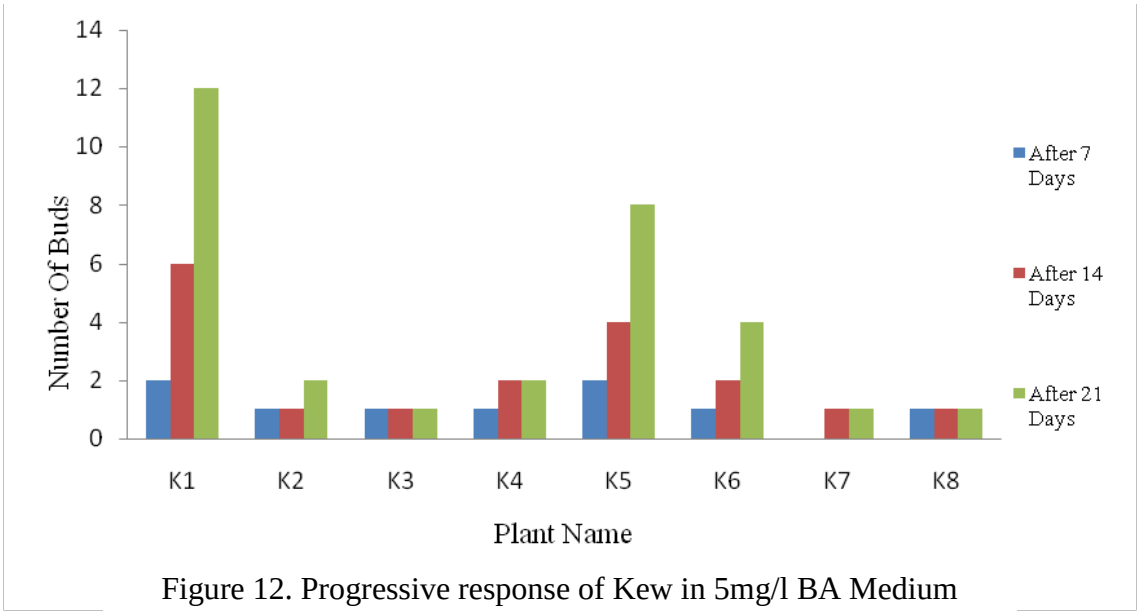


Figure 12. Progressive response of Kew in 5mg/l BA Medium

Table 8. Periodical Response of Kew Callus Initiation

Plant Name	*After 7 Days*	*After 14 Days*	*After 21 Days*
K1	Callusing	Minimum Callus	Minimum callus
K2	Minimum Callus	Medium Callus	Medium Callus
K3	Minimum Callus	Medium Callus	Medium Callus
K4	Callusing	Minimum Callus	Medium Callus
K5	Minimum Callus	Medium Callus	Medium Callus
K6	Callusing	Medium Callus	Medium Callus
K7	Minimum Callus	Minimum Callus	Minimum callus
K8	Minimum Callus	Medium Callus	Medium Callus

1.2 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 n with farmers' 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 and established at the center will be evaluated for their growth, yield and quality characteristics and a suitable yield index will be developed involving Pineapple Farmers' Association. The 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.

Altogether 11 pineapple types are being evaluated in RBD with 3 replications in the field.

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 H5 and MD-2 recorded higher growth parameters.

Observation taken after 8 months also witnessed Mauritius with the highest values for all growth parameters and was significantly superior to all other accessions. The best performance of Mauritius can be 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 the poorest for H2 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. No. 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 no. of leaves was highest in H4 followed by MD-2 which was on par.

Table 9. Growth Parameters of Pineapple Accessions 24 Months after Planting

No	Accessions	Plant height (cm)	Canopy spread (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)
1	Mauritius	90.07	121.33	31.20	70.33	5.03
2	Kew	110.07	129.93	62.17	82.07	4.87
3	MD-2	77.73	115.47	25.87	64.40	4.73
4	MTS	71.13	101.27	24.53	55.60	4.03
5	T_3	94.83	120.17	47.22	74.47	5.13
6	H_1	54.40	77.40	23.80	43.73	4.63
7	H_2	40.28	53.54	16.61	32.99	2.63
8	H_3	89.07	109.93	36.00	71.00	5.03
9	H_4	77.52	96.40	57.07	46.40	5.10
10	H_5	85.93	98.53	34.40	69.20	4.37
11	Amrutha	80.33	97.00	39.47	59.00	4.40
	GM	79.21	101.91	36.21	60.84	4.54
	SEM	3.735	5.609	2.22	3.607	0.307
	CD (0.05)	11.019	16.546	6.55	10.642	0.905
	CV%	8.167	9.533	10.624	10.271	11.700

At 24 months after planting, the pineapple accessions showed statistically significant variations in all the growth parameters observed. Kew recorded maximum plant height of 110.07 cm which was significantly higher than that recorded by others. Kew was followed by T3, Mauritius, H3 and H5. H2 recorded the lowest plant height of 40.28 cm followed by H1 and MTS. Canopy spread was highest for Kew which was statistically on par with Mauritius, T3 and MD-2. The lowest canopy spread was recorded by H2 followed by H1 which were significantly inferior to all others. Kew followed by H4 recorded highest number of leaves and they were on par. H2 followed by H1 recorded least number of leaves. Leaf length was highest for Kew which was on par with T3 and significantly superior to all others. H2 followed by H1 recorded lowest leaf length.

Leaf width was highest for T3 followed by H4 and Mauritius which were all on par. H2 followed by MTS recorded lowest leaf width. In general Kew recorded highest growth parameters except leaf width. H2 and H1 recorded least growth parameters at 24 months after planting.

Table 10. Growth Parameters of Pineapple Accessions 28 Months after Planting

No	Accessions	Plant height (cm)	Canopy spread (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)
1	Mauritius	96.20	108.07	39.87	79.20	4.47
2	Kew	111.00	121.80	73.60	69.00	5.10
3	MD-2	80.73	101.33	29.40	61.33	4.60
4	MTS	91.87	102.53	34.40	63.67	4.27
5	T_3	88.63	114.33	40.00	59.30	4.83
6	H_1	64.00	88.07	29.00	53.27	5.10
7	H_2	49.22	69.50	23.93	38.37	2.83
8	H_3	61.73	104.00	31.93	54.00	4.03
9	H_4	77.67	89.00	56.23	43.53	4.30
10	H_5	84.67	114.67	33.87	56.07	4.40
11	Amrutha	77.00	100.27	40.00	54.27	4.17
	GM	80.25	101.23	39.30	58.37	4.37
	SEM	5.055	6.069	5.086	4.110	0.186
	CD (0.05)	14.912	17.905	15.005	12.126	0.549
	CV%	10.910	10.384	22.415	12.197	7.367

At 28 months after planting, all the growth parameters recorded significant variations among the accessions. The plant height was maximum of 111 cm for Kew followed by Mauritius. The variety Kew was superior to all other types in plant height. H2 and H3 were on par and significantly inferior to all others in plant height. Canopy spread was highest of 121.80 cm for Kew which was the highest, followed by H5, T3, Mauritius and H3 which were all on par. H2 recorded the lowest canopy spread and it was significantly inferior to all others in canopy spread. Kew variety was recorded to have the highest number of leaves followed by H4 which were significantly different and superior to all other varieties. H2 was again lowest in the number of leaves. Leaf Length was highest for Mauritius followed by Kew, which were on par and was

significantly superior to all other varieties. H2 was on par with H4 and significantly inferior to all other types in leaf length. Leaf width was highest of 53.27 cm for H1 followed by Kew, T3 and MD-2 which were all on par. H1 and Kew were superior to the rest of the accessions in leaf width. H2 recorded the minimum leaf width and it was significantly inferior to all other pineapple accessions. In general the variety Kew recorded higher growth parameters and H2 was poorest in growth performance.

Table 11. Growth Parameters of Pineapple Accessions 32 Months after Planting

No	Accessions	Plant height (cm)	Canopy spread (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)
1	Mauritius	75.73	91.27	35.93	61.13	3.70
2	Kew	111.07	108.13	99.27	65.87	4.63
3	MD-2	87.87	99.20	33.00	64.07	3.93
4	MTS	79.07	90.60	36.33	58.87	3.53
5	T_3	83.40	90.97	58.20	54.33	4.40
6	H_1	71.80	87.93	35.47	56.13	4.87
7	H_2	53.25	73.83	25.33	43.67	3.27
8	H_3	78.73	94.67	28.67	48.40	4.30
9	H_4	83.47	79.13	53.97	42.40	4.30
10	H_5	87.07	102.20	33.93	61.00	4.10
11	Amrutha	64.60	62.53	30.07	39.20	3.67
	GM	79.64	89.12	42.74	54.10	4.06
	SEM	5.016	5.880	7.003	3.645	0.182
	CD (0.05)	14.799	17.347	20.659	10.753	0.536
	CV%	10.910	11.429	28.379	11.671	7.750

At 32 months after planting, Kew recorded maximum plant height of 111. 07 cm which was significantly superior to all other pineapple accessions, which was followed by MD-2, H5 H4, and T3. H2 was recorded to have the minimum plant height followed by Amrutha and H1. Canopy spread was the highest of 108.13 cm of Kew which was followed by H5, MD-2, H3, Mauritius and T3 , which were all on par. Amrutha had the lowest canopy spread followed by H2 and H4 which were all on par. Kew recorded the maximum number of leaves and it was

significantly superior to all other accessions, which was followed by T3 andH4. H2 followed by H3 AND Amrutha recorded the lowest number of leaves which were all on par. Leaf length was the maximum of 65.87 cm for Kew followed by MD-2, Mauritius, H5, MTS and H1, which were all on par. Minimum leaf length was recorded by Amrutha followed by H4, H2and H3 which were all on par and inferior to the rest of the varieties. Leaf width was the maximum of 4.87 cm for H1 followed by Kew and T3 which were on all par. H2 followed by MTS Amrutha and Mauritius had lower leaf width and they were all on par and inferior to the rest of the varieties in leaf width. In general Kew had the best growth performance while H2 and Amrutha had poor growth performance at 32 months after planting.

Fruits obtained from various accession numbers were analyzed for yield characters, phytochemical characters and qualitative characters. Yield character studies included calculation of fruit weight with crown, crown weight, fruit weight, peel weight, core weight, juice weight, pulp weight, fruit length, pulp diameter, core diameter, stock length and stock diameter. Phytochemical analysis quantified the TSS, pH, acidity, presence of ascorbic acid, reducing sugars, non- reducing sugars and total sugar in percentages. Taste, colour, size and aroma of the fruits were studied qualitatively.

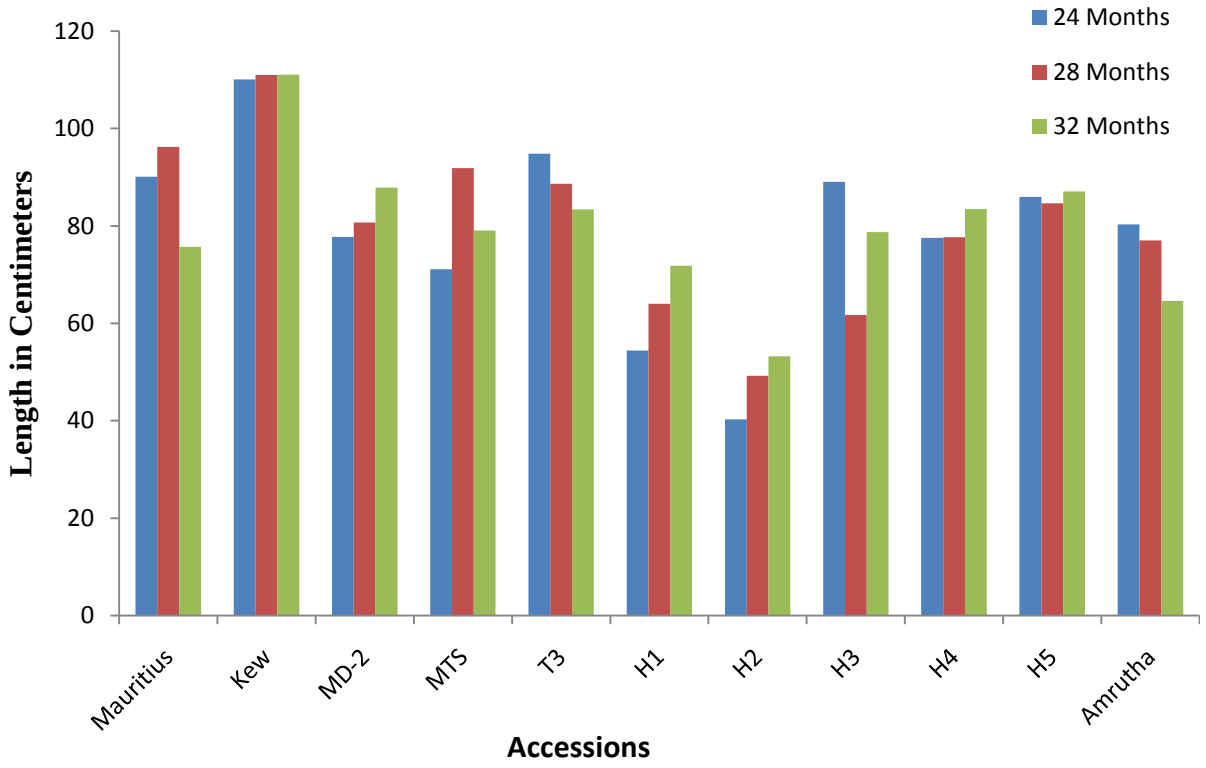


Figure 13. Comparison of Plant height of 11 Pineapple Acessions

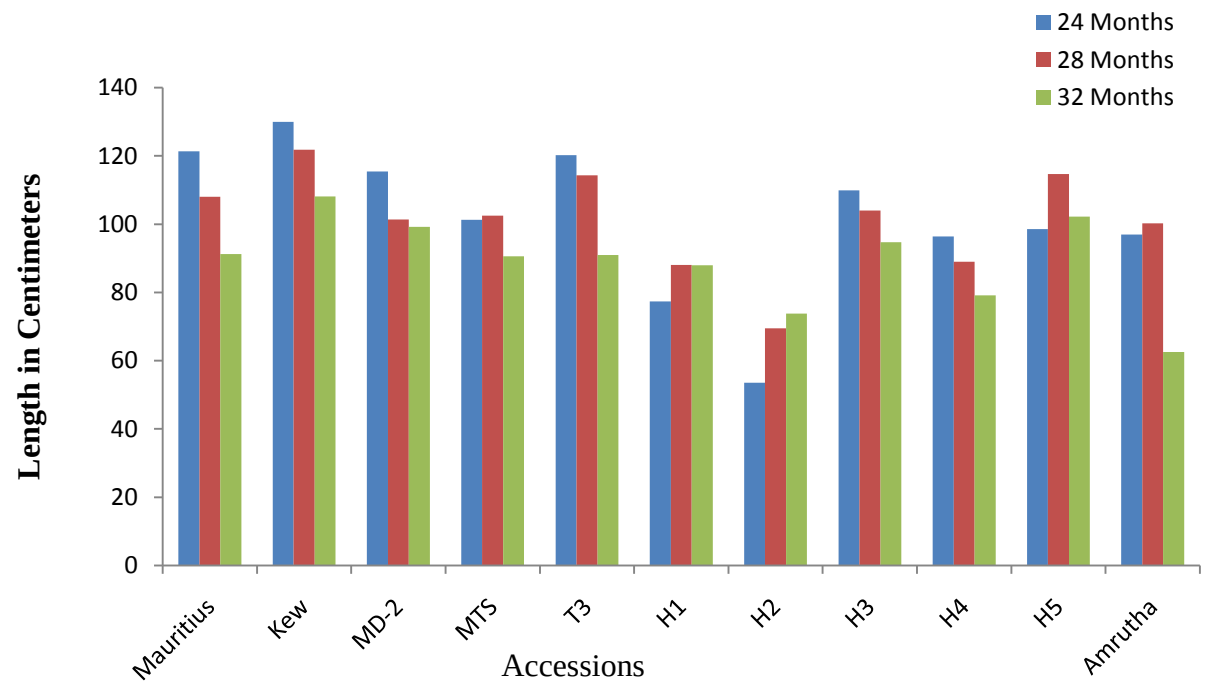


Figure 14. Comparison of Canopy spread of 11 Pineapple Acessions

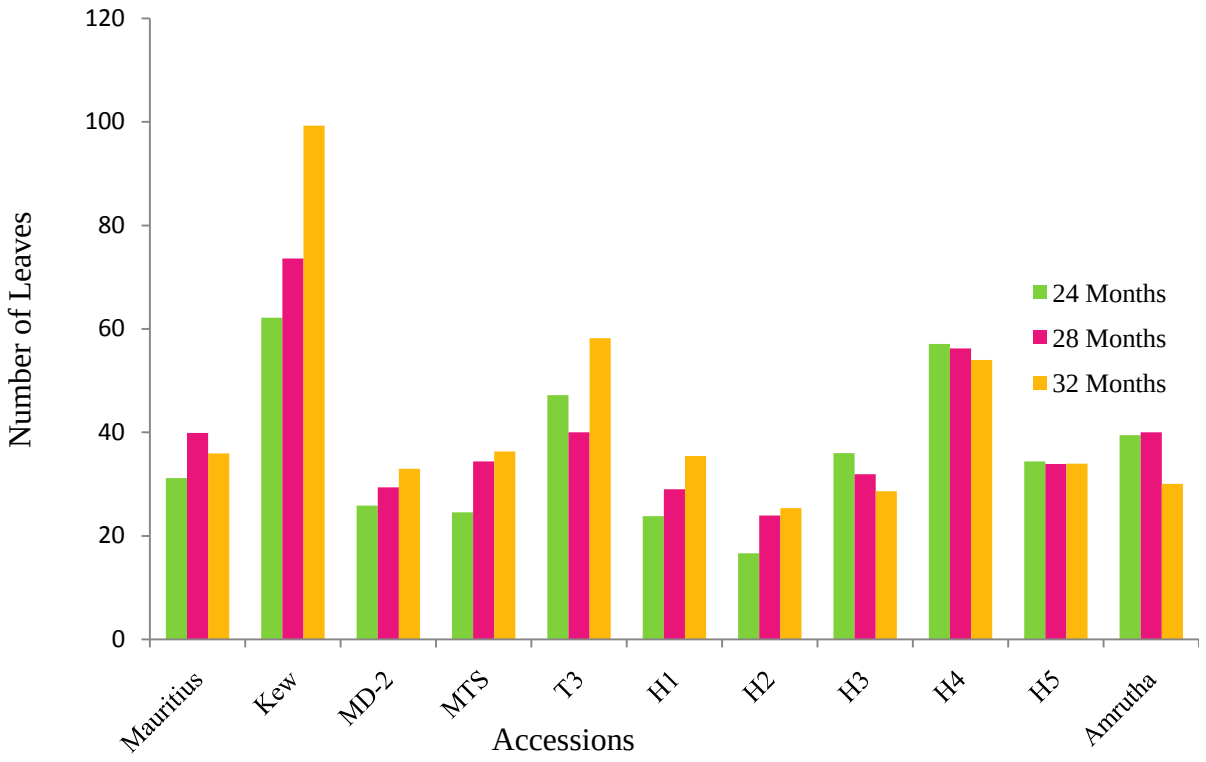


Figure 15. Comparison of Total Number of Leaves of 11 Pineapple Acessions

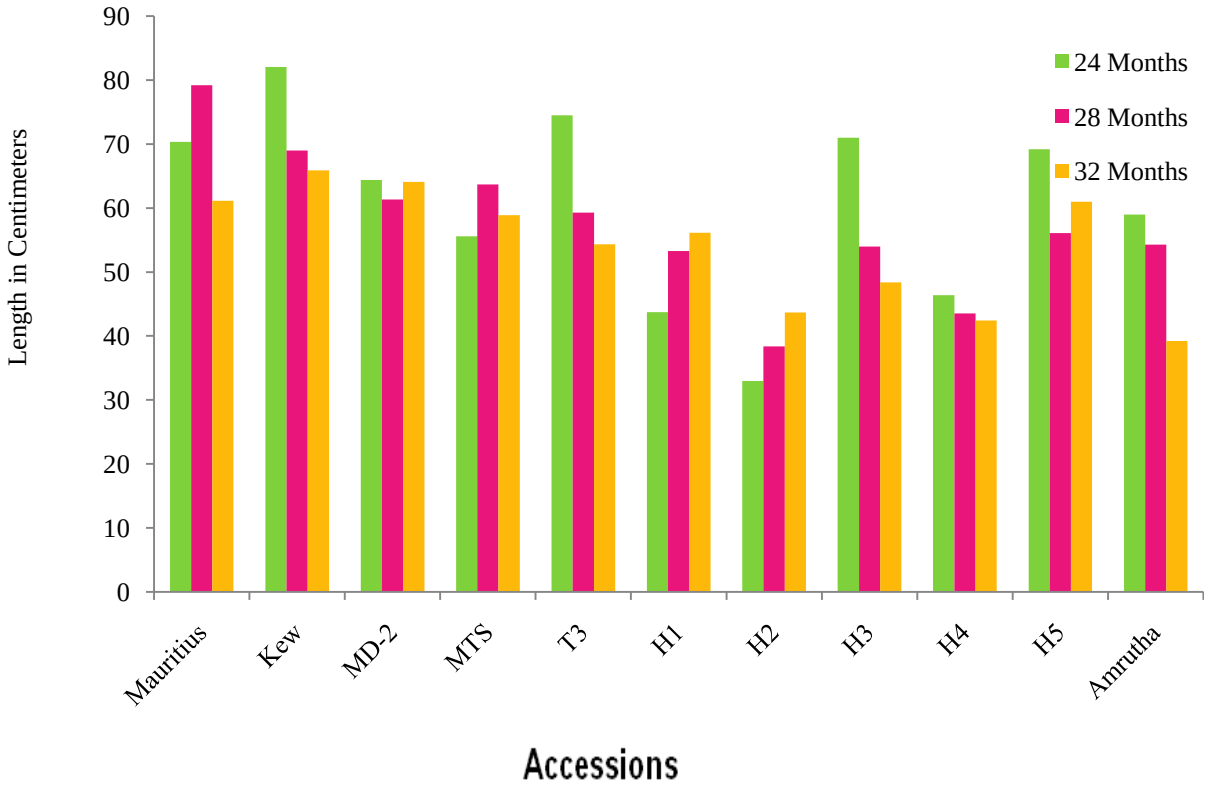


Figure 16. Comparison of Leaf Length of 11 Pineapple Acessions

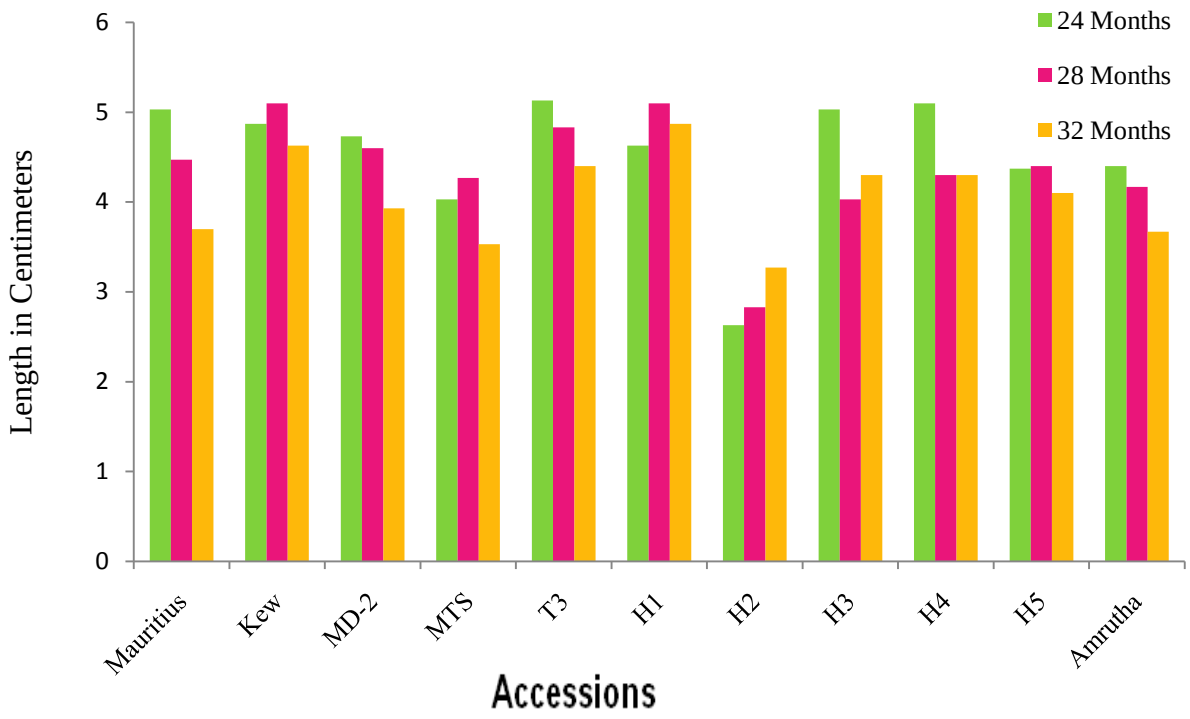


Figure 17. Comparison of Leaf width of 11 pineapple Acessions

Table 12. Year-wise fruit number and weight/plot and mean fruit weight of pineapple types

Pineapple	Fruit number/plot				Fruit weight/plot (g)				Mean fruit weight (g)			
	I Year	II Year	III Year	Total	I Year	II Year	III Year	Total	I Year	II Year	III Year	Mean
MAURITIUS	1.33	13.00	4.67	19.00	1984.67	17183.67	5009.17	24177.50	1488.50	1321.82	1073.39	1294.57
KEW			1.67	1.67			5147.00	5147.00			3088.20	1029.40
MD2		3.00	3.67	6.67		5359.50	6654.17	12013.67		1786.50	1814.77	1200.42
MTS		3.00	2.33	5.33		3379.83	3440.33	6820.16		1126.61	1474.43	867.01
T3		1.00	5.67	6.67		2603.00	15498.00	18101.00		2603.00	2734.94	1779.31
H1			0.67	0.67			920.00	920.00			1380.00	460.00
H2												
H3			3.67	3.67			5875.17	5875.17			1602.32	534.11
H4		1.00	2.00	3.00		2265.50	4222.83	6488.33		2265.50	2111.42	1458.97
H5			4.00	4.00			5808.67	5808.67			1452.17	484.06
AMRUTHA			2.67	2.67			3462.50	3462.50			1298.44	432.81

Third year completes only on 31.05.2013 but the data is as on 31.03.2013

Table 13. Year-wise fruit number and weight/ha and mean fruit weight of pineapple types

	Fruit number/ha				Fruit weight/ha (kg)			
Pineapple	I Year	II Year	III Year	Total	I Year	II Year	III Year	Total
MAURITIUS	6584	64198	23045	93827	9801	84858	24737	119395
KEW			8230	8230			25417	25417
MD2		14815	18107	32922		26467	32860	59327
MTS		14815	11523	26337		16691	16989	33680
T3		4938	27984	32922		12854	76533	89388
H1			3292	3292			4543	4543
H2								
H3			18107	18107			29013	29013
H4		4938	9877	14815		11188	20853	32041
H5			19753	19753			28685	28685
AMRUTHA			13169	13169			17099	17099

Third year completes only on 31.05.2013 but the data is as on 31.03.2013

Mauritius started yielding from first year. T3, MD-2, MTS and H4 started yielding from second year only. H3, H5, Kew, Amrutha and H1 started yielding from third year. H2 has not yet started yielding. Yield data obtained so far shows that Mauritius is the most superior followed by T3 and MD-2 in yield.

Table 14. Mean Value of Yield Characters of Pineapple Accessions

No	Accessions	Stock Length (cm)	Stock Diameter (cm)	Fruit Length (cm)	Fruit + Crown(g)	Crown Weight(g)	Fruit wt(g)
1	Mauritius	10	2.5	53	1543	149.5	1360.5
2	Kew	6.8	3.4	44.3	3445	246.3	3198.7
3	MD-2	7.3	3.7	37.5	1996.5	162	1834.5
4	MTS	7.5	2.5	32.33	1407.5	85	1371.3
5	T_3	8.73	2.8	44.5	2242	236.33	2005.7
6	H_1	6.8	3.2	32	1018	281	737
7	H_2	-	-	-	-	-	-
8	H_3	4.6	2.4	43.5	1417.33	321.33	1096
9	H_4	2.5	2.26	31	2173.17	121	913.5
10	H_5	9.06	2.26	38	1034.5	121	913.5
11	Amrutha	6.3	2.4	33	1297.33	83	1242

*Fruiting of Accession H2 is yet to happen

Table 15. Mean Value of Yield Characters of Pineapple Accessions

No.	Accessions	Peel wt(g)	Core wt(g)	Core Diameter (cm)	Pulp wt(g)	Pulp Diameter (cm)	Juice wt(g)
1	Mauritius	208.75	119.5	3	850	9.25	546.5
2	Kew	432.7	344	3.8	2174.6	5.5	1524.3
3	MD-2	135	126	2.7	1208	5	809.83
4	MTS	100	141.67	2.7	814	4.2	583.3
5	T_3	213.33	230.67	3.16	1228.6	5.13	851
6	H_1	205	151	2.8	466	3.9	583
7	H_2	-	-	-	-	-	-
8	H_3	171.33	133.67	2.4	189.66	4.77	375
9	H_4	309	112	2.6	1446.6	5.03	336.17
10	H_5	309	112	2.3	497.6	3.1	336.17
11	Amrutha	226	117	2.17	224	4.17	534

*Fruiting of Accession H2 is yet to happen

Table 16. Mean Value of Phytochemical Characters of Pineapple Accessions

No	Accessions	TSS	pH	Acidity	Ascorbic acid	Reducing sugar	Non red. Sugar	Total sugar
1	Mauritius	19.76	3.25	0.745	67.51	2.19	12.79	14.99
2	Kew	13.9	3.54	0.6	32.32	3.53	11.57	15.61
3	MD-2	18.4	3.47	0.75	54.52	3.57	10.91	14.58
4	MTS	18.9	3.77	0.67	48.59	3.03	8.18	11.49
5	T_3	15.1	3.85	0.60	48.48	4.67	9.83	15.02
6	H_1	16.8	3.33	1.1	72.72	3.91	13.25	17.86
7	H_2	-	-	-	-	-	-	-
8	H_3	17.2	3.62	0.36	38.38	4.11	10.58	15.24
9	H_4	17.9	3.72	0.43	46.46	4.92	11.14	16.65
10	H_5	18.67	3.74	0.52	42.42	4.74	12.43	17.83
11	Amrutha	14.07	3.72	0.58	38.4	3.13	13.84	17.71

*Fruiting of Accession H2 is yet to happen

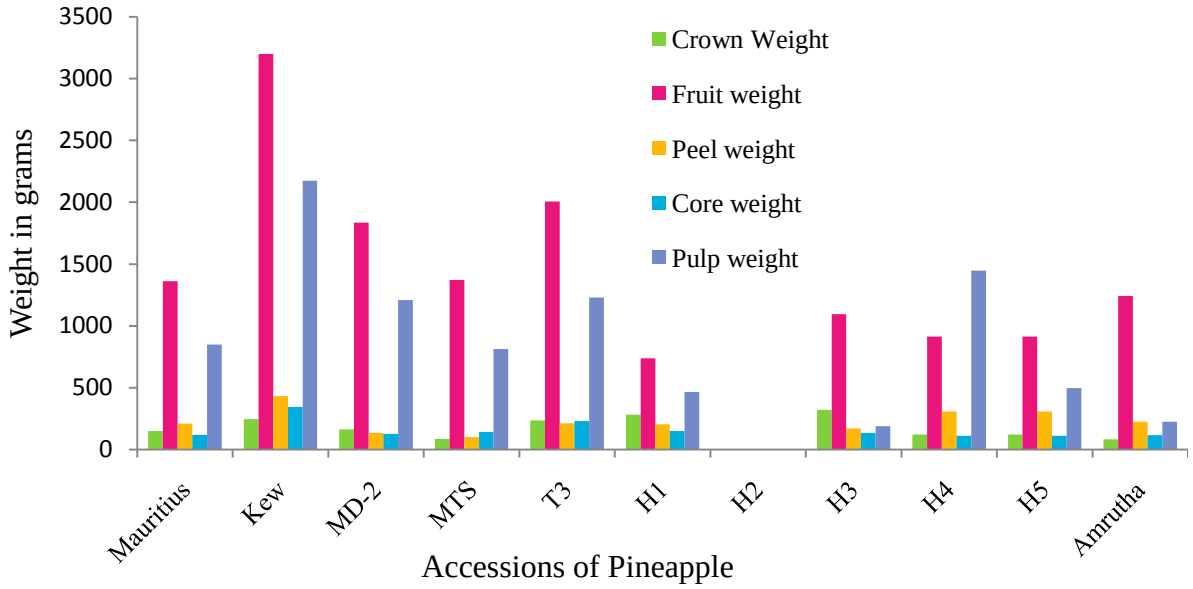


Figure 18. Comparison on Yield Characters of 11 Pineapple Accessions

Table 17. Mean Value of Qualitative Characters of Pineapple Accessions (0 – 9 Scale)

No.	Accessions	Taste	Colour	Size	Smell	Pulp Colour	Juice Colour
1	Mauritius	4.5	4	4	4.5	4	4
2	Kew	3.5	3.2	6	2.8	3.8	4.2
3	MD-2	3.7	4.5	7.3	4.7	4.2	4.6
4	MTS	4.6	4.83	5.3	3.8	4.6	5
5	T_3	2.6	2.7	5	2.3	2.67	2.67
6	H_1	5	3	3	3	3	3
7	H_2	-	-	-	-	-	-
8	H_3	4.2	3.33	3	3	3.67	3.67
9	H_4	4.3	4.3	5.2	4	4	4
10	H_5	3.7	3.8	2.8	4	3.6	3.3
11	Amrutha	3.33	4.33	4.33	3.5	4.83	4.17

*Fruiting of Accession H2 is yet to happen

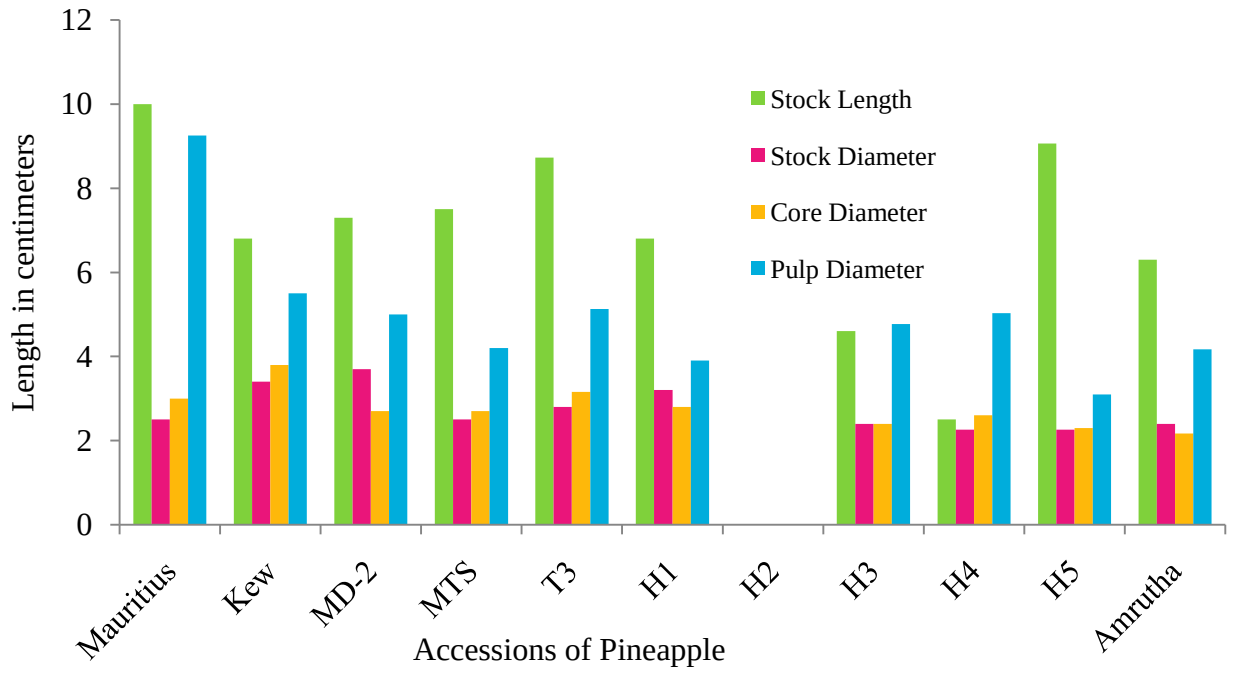


Figure 19 . Comparison on Yield Characters of 11 Pineapple Accessions

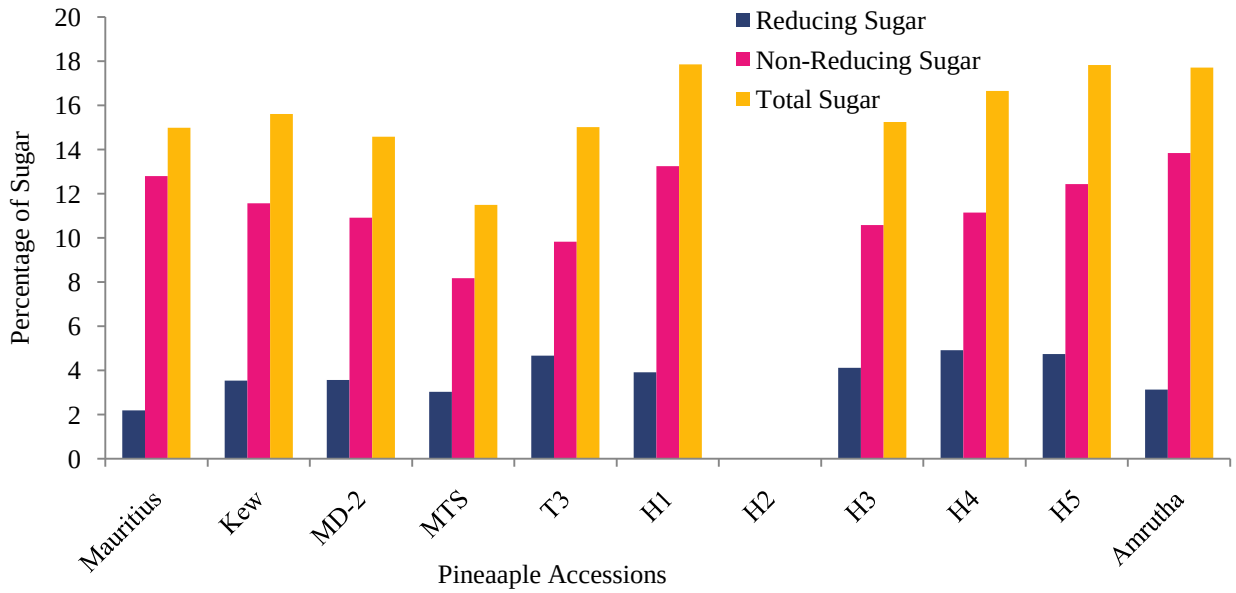


Figure 20. Comparison of sugar content of Pineapple Accessions

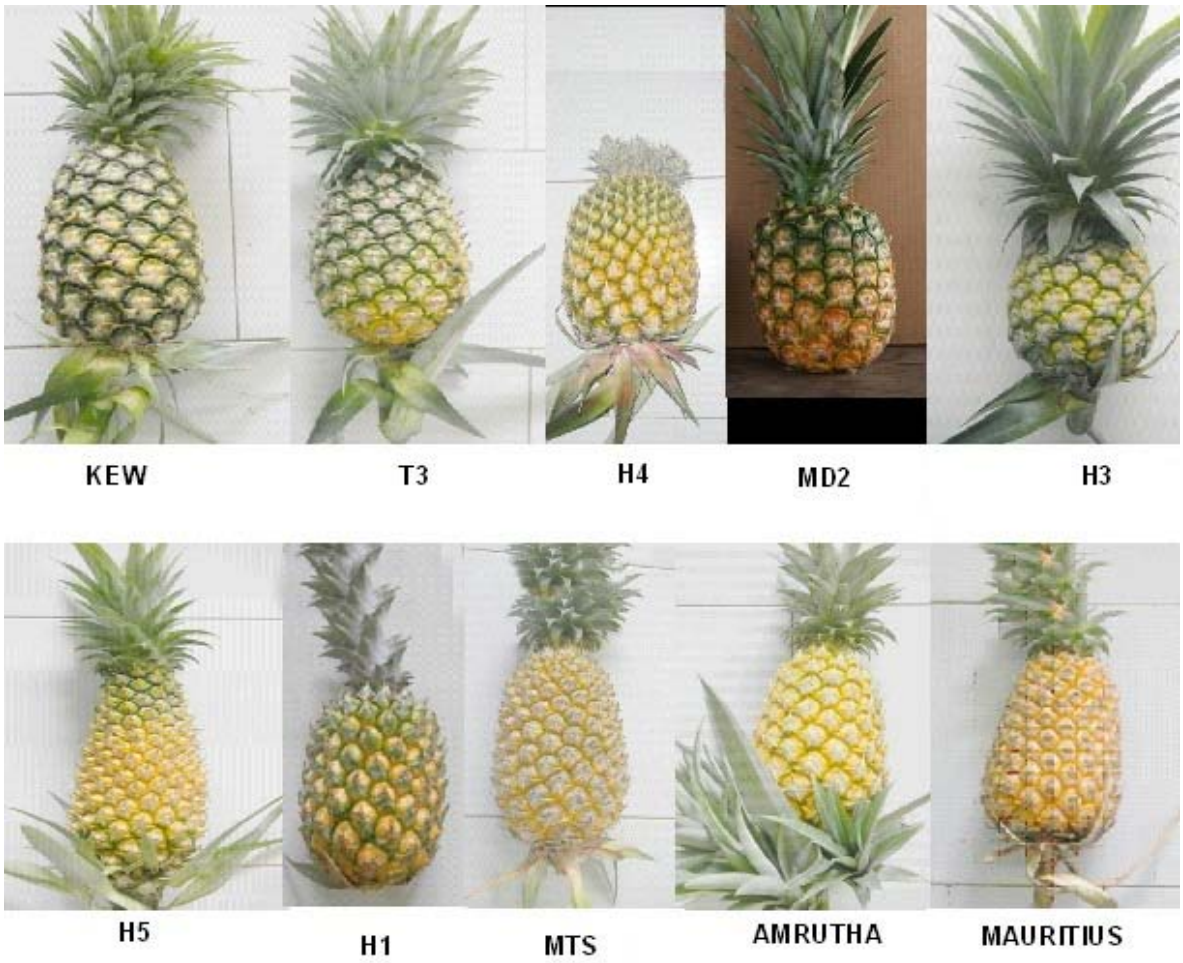


Figure 21. Fruits of various pineapple types

1.2.1 Shelf Life Studies of Pineapple Variety MD-2

Objective

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

Technical Programme

Shelf life studies were done by keeping the harvested fruits in room temperature for 9 days and were observed every 3 days interval.

Results

Tabulated observations of the yield characters, phytochemical characters and qualitative characters of different accessions of MD2 variety is furnished below.

Table 18. Periodical changes in yield characters of MD2 pineapple during shelf life studies

Days	Fruit+ crown wt(g)	Crown wt. (g)	Fruit wt(g)	Peel wt. (g)	Core wt. (g)	Juice wt. (g)	Pulp wt (g)
0	1696.5	231	1465.5	163	136	750	1251
3	1940	468	1472	261	161	985	1119
6	1647.5	124.5	1523	228	100	394.5	1182
9	1656	286	1570	197	179	775	1173

Table 19. Periodical percentagewise changes in the yield characters of MD2 pineapple during shelf life studies

Days	Fruit+ crown wt (%)	Crown wt. (%)	Fruit wt (%)	Peel wt. (%)	Core wt. (%)	Juice wt. (%)	Pulp wt (%)
0	100	13.62	86.38	9.61	8.02	44.21	73.74
3	100	24.12	75.88	13.45	8.30	50.77	57.68
6	100	7.56	92.44	13.84	6.07	23.95	71.75
9	100	17.27	94.81	11.90	10.81	46.80	70.83

Table 20. Periodical changes in phytochemical characters of MD2 pineapple during shelf life

Days	TSS (%)	pH	Acidity (%)	Reducing sugar (%)	Non red. sugar (%)	Total sugar (%)	Ascorbic acid (mg/100g)
0	15.2	3.62	0.51	3.52	8.53	12.5	72.72
3	16.2	3.32	0.51	6.52	8.91	15.63	72.72
6	15.8	3.55	0.64	5.68	15.81	22.32	60.60
9	15	3.68	0.83	6.57	16.16	23.58	72.72

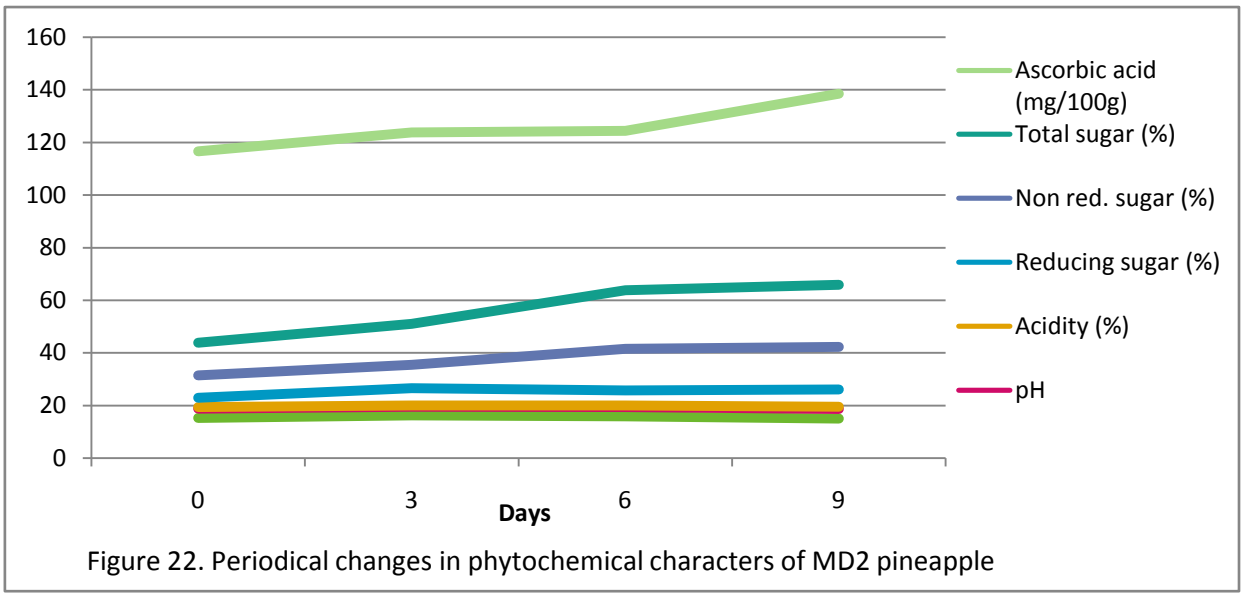


Figure 22. Periodical changes in phytochemical characters of MD2 pineapple

Table 21. Periodical changes in qualitative characters of MD2 pineapple (0-9) scale during shelf life

Days	Taste	Colour	Smell
0	5	5	4.5
3	6	5	6
6	5	6	6
9	5	5	5

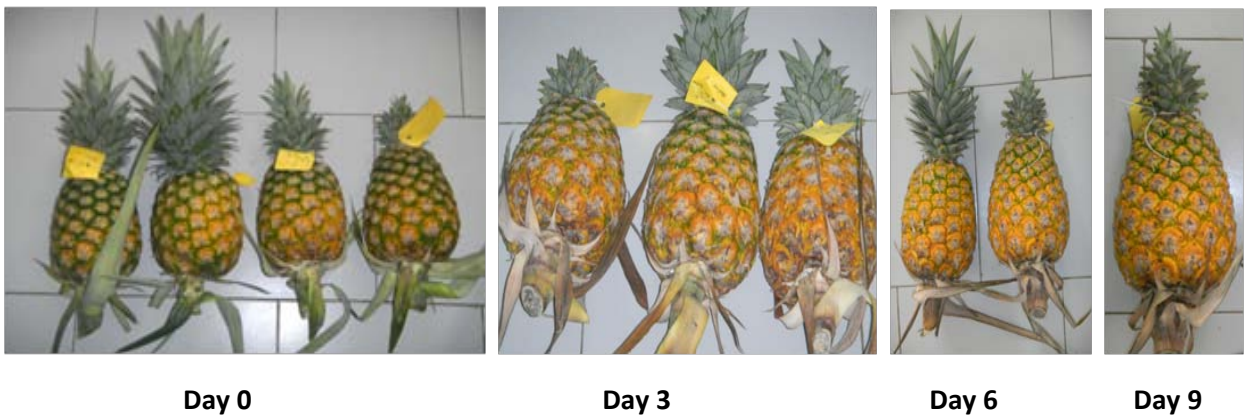


Figure 23. Periodical changes in MD2 pineapple variety during shelf life

1.3 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 22. PRS 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

1.3.1 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.

Table 23. Descriptive yield statistics of Pineapple Accessions as on March 2013

Statistic	Fruit+Crown wt (g)	Crown wt (g)	Peel wt (g)	Core wt (g)	Pulp wt (g)	Fruit wt (g)	Juice wt (g)
Mean	1295.97	166.70	184.59	137.30	742.14	1131.80	459.95
Standard Error	33.51	9.34	4.98	4.60	18.04	29.76	12.17
Median	1201.75	125.50	172.00	116.50	714.00	1095.50	436.00
Mode	609.00	17.00	175.00	113.00	544.00	915.50	530.00
Std Deviation	595.73	166.09	88.18	81.45	312.94	528.99	214.57
Sample Variance	354890.88	27586.48	7774.99	6633.46	97930.40	279827.41	46038.62
Kurtosis	0.91	10.92	4.91	3.54	1.68	1.37	2.40
Skewness	0.89	2.75	1.65	1.58	0.93	0.97	1.15
Range	3099.50	1304.00	649.00	500.50	1983.50	2734.00	1367.50
Minimum	235.50	0.00	34.00	20.50	139.50	226.00	67.50
Maximum	3335.00	1304.00	683.00	521.00	2123.00	2960.00	1435.00
Sum	409527.95	52678.50	57777.80	42973.50	223384.50	357648.45	143043.00
Count	316.00	316.00	313.00	313.00	301.00	316.00	311.00

Table 24. Descriptive quality statistics of Pineapple Accessions as on March 2013

Statistic	TSS (%)	PH	Acidity (%)	Ascorbic Acid	Reducing Sugar (%)	Non Red Sugar (%)	Total Sugar (%)	Taste (0-9 score)	Colour (0-9 score)	smell (0-9 score)
Mean	19.60	3.82	0.19	33.73	5.36	21.28	27.59	3.90	3.50	2.85
Std Error	0.18	0.02	0.01	0.93	0.07	0.36	0.40	0.07	0.05	0.04
Median	19.40	3.82	0.12	30.30	5.17	21.72	28.08	4.00	3.00	3.00
Mode	19.00	3.78	0.07	30.30	5.43	25.53	27.17	3.00	3.00	3.00
Std Devn	3.18	0.26	0.19	15.58	1.25	6.03	6.71	1.28	0.87	0.79
Variance	10.08	0.07	0.04	242.59	1.56	36.40	45.06	1.64	0.76	0.62
Kurtosis	1.16	0.26	2.92	1.76	3.33	0.64	1.06	-0.49	0.99	-0.13
Skewness	0.04	0.53	1.98	1.10	1.12	0.02	-0.23	0.38	0.84	0.68
Range	21.60	1.55	0.92	90.90	9.66	38.35	48.22	7.00	5.00	3.00
Minimum	8.40	3.27	0.01	6.06	2.24	6.09	3.86	1.00	2.00	2.00
Maximum	30.00	4.82	0.93	96.96	11.90	44.44	52.08	8.00	7.00	5.00
Sum	6193.00	1082.10	52.73	9546.82	1517.28	6021.58	7807.49	1214.10	1090.00	886.80
Count	316.00	283.00	283.00	283.00	283.00	283.00	283.00	311.00	311.00	311.00

Table 25. Yield Characters of Pineapple Accessions as on March 2013

Sl.No	Plant No.	Weight with Crown (g)	Crown weight (g)	Fruit weight (g)	Pulp weight (g)	Peel Weight (g)	Core weight (g)	Juice weight (g)
1	264	2246.50	210.50	2036.00	1326.33	266.33	318.00	876.33
2	1113	1530.50	53.00	1477.50	520.00	202.00	200.00	848.00
3	8773	1568.50	125.00	1443.50	1075.00	203.00	167.00	578.00
4	12488	2232.00	801.25	1430.75	710.50	134.25	174.75	509.75
5	6	1733.00	309.00	1424.00	484.50	108.50	71.50	437.00
6	2982	1445.50	31.50	1414.00	987.00	223.00	112.00	613.00
7	8689	1473.00	78.00	1395.00	772.00	178.00	111.00	704.00
8	5165	1736.25	409.75	1326.50	907.50	172.50	129.50	550.00
9	2770	1526.25	225.00	1301.25	869.50	168.50	134.50	563.50
10	743	1868.75	578.25	1290.50	626.00	175.00	181.50	505.50
11	982	1400.50	133.33	1267.17	870.67	134.67	166.67	592.00
12	11-B	1326.50	60.50	1266.00	901.00	224.00	178.00	547.00
13	10952	1509.33	269.00	1240.33	852.67	207.33	169.00	497.67
14	3318	1258.33	83.33	1175.00	905.33	170.67	95.67	530.00
15	7058	1247.50	92.50	1155.00	804.00	132.00	180.00	532.00
16	6028	1244.00	115.00	1129.00	661.00	207.00	85.00	407.00
17	3946	1325.50	202.50	1123.00	894.00	122.00	106.00	600.00
18	2774	1320.67	221.67	1099.00	667.33	146.50	124.17	489.00
19	775	1393.58	300.96	1092.63	761.08	143.46	130.29	384.07
20	11A	1198.25	110.50	1087.75	661.00	329.50	130.50	448.00
21	2913	1423.94	341.75	1082.19	610.13	224.94	184.41	394.94
22	3467	1223.40	169.30	1054.10	720.80	156.80	130.80	456.20
23	5946	1146.00	126.50	1019.50	351.00	56.50	57.00	341.00
24	461	1144.06	176.81	967.25	619.19	159.44	157.19	469.00
25	2520	953.00	26.50	926.50	541.00	221.00	109.00	344.00

Table 26. Phytochemical Characters of Pineapple Accessions as on March 2013.

Sl. No	Plant No.	TSS (%)	PH	Acidity (%)	Ascorbic Acid (mg/100g)	Reducing Sugar (%)	Non Red Sugar (%)	Total Sugar (%)
1	264	20.60	3.77	0.13	52.52	4.54	20.32	25.93
2	1113	20.20	3.64	0.17	12.12	6.32	20.31	27.77
3	8773	20.40	3.69	0.05	30.30	4.62	27.24	33.30
4	12488	21.00	3.82	0.29	45.48	5.41	14.98	21.18
5	6	23.00	4.13	0.05	12.12	8.47	11.41	20.49
6	2982	22.00	3.55	0.15	42.42	4.90	38.52	45.45
7	8689	21.60	3.27	0.21	54.54	4.54	26.93	32.89
8	5165	21.20	3.91	0.09	33.33	5.46	19.42	25.91
9	2770	23.20	3.82	0.18	9.09	8.25	19.75	29.02
10	743	21.00	3.82	0.11	19.70	4.65	21.72	27.52
11	982	20.60	3.90	0.12	22.22	5.95	23.57	30.79
12	11-B	20.00	3.85	0.07	36.36	5.43	24.21	30.92
13	10952	20.00	3.78	0.10	36.36	5.10	23.30	29.44
14	3318	20.67	3.52	0.39	38.38	5.00	17.60	23.52
15	7058	23.00	3.74	0.08	18.18	4.50	21.53	27.17
16	6028	23.00	3.92	0.08	48.48	5.10	16.35	22.32
17	3946	20.60	3.52	0.19	54.54	4.46	23.37	29.06
18	2774	20.67	3.90	0.10	26.26	5.47	23.31	26.84
19	775	20.87	2.20	0.28	33.76	6.78	16.90	24.58
20	11A	22.10	3.63	0.38	22.71	4.53	16.95	22.38
21	2913	22.34	3.89	0.16	19.19	5.56	22.15	24.37
22	3467	22.16	3.94	0.09	24.24	5.35	25.83	32.73
23	5946	20.00	3.95	0.08	24.24	5.68	15.42	21.92
24	461	20.55	3.48	0.10	38.09	5.83	27.27	34.54
25	2520	28.20	4.31	0.11	18.18	4.90	21.72	27.77

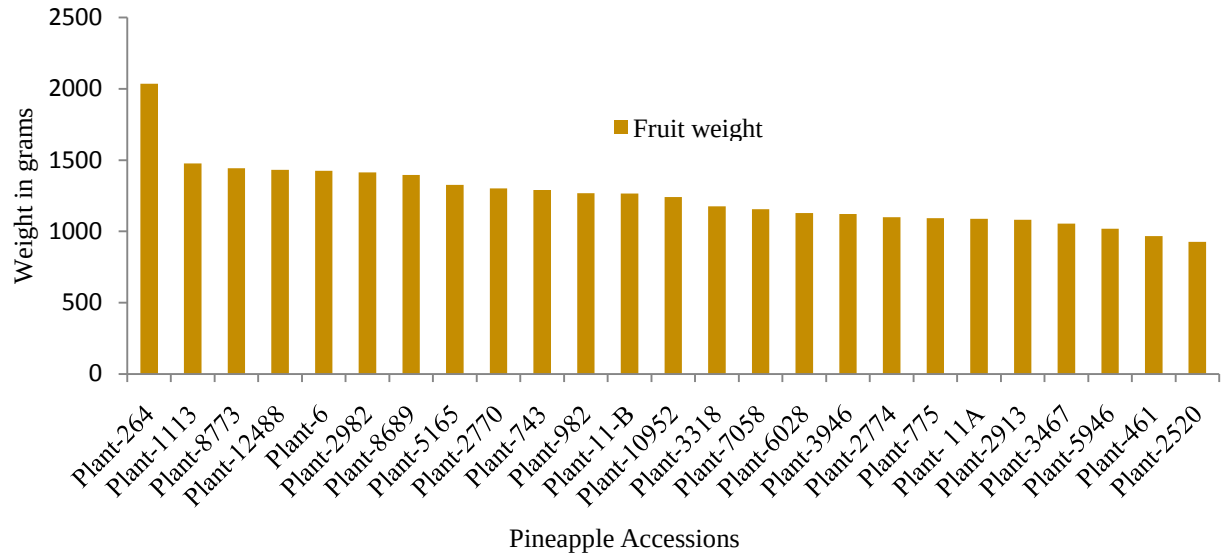


Figure 24. Comparison of Fruit weight of pineapple varieties

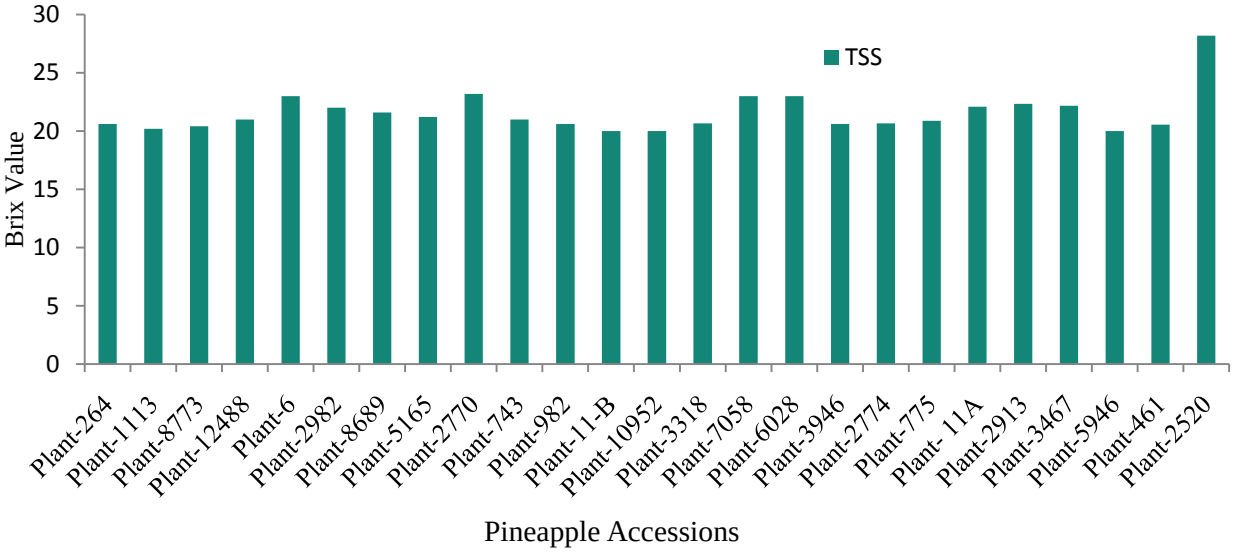


Figure 25. Comparison of TSS of pineapple varieties

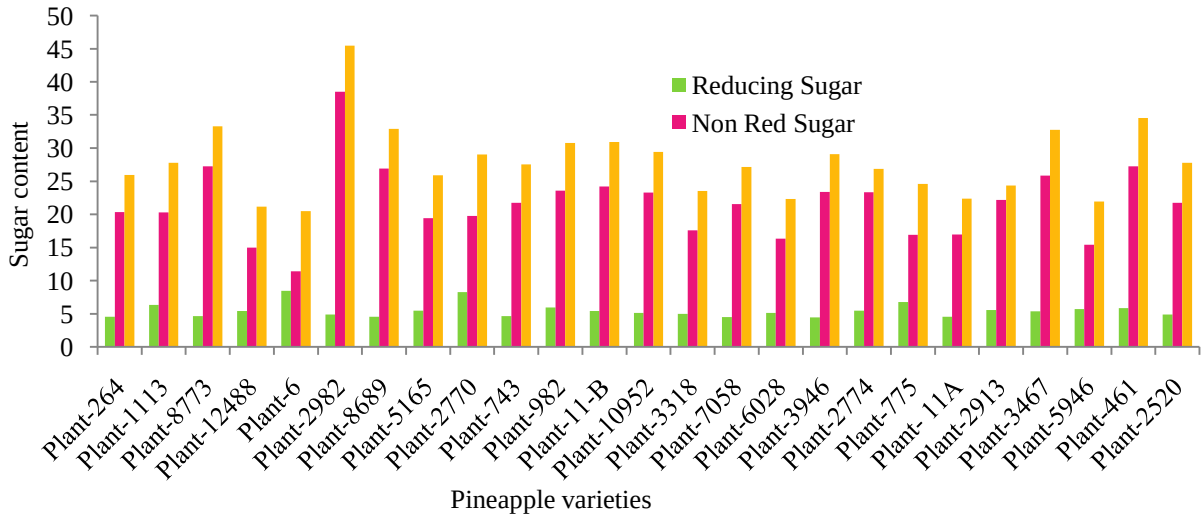


Figure 26. Comparison of sugar contents of pineapple varieties

Table 27. Qualitative Characters of Pineapple Accessions as on March 2013(0 – 9 Scale)

Sl.	Plant	Taste	Colour	Size
1	264	4.33	3.00	4.67
2	1113	6.00	4.00	3.50
3	8773	3.50	5.00	5.00
4	12488	5.25	5.00	4.00
5	6	2.50	4.00	3.00
6	2982	5.00	5.00	4.00
7	8689	5.00	3.50	5.50
8	5165	4.00	4.50	4.75
9	2770	6.50	4.00	4.50
10	743	5.00	4.00	4.00
11	982	4.83	3.17	3.83
12	11-B	4.00	3.00	4.00
13	10952	4.00	5.00	4.33
14	3318	4.93	4.00	3.77
15	7058	6.00	4.00	5.00
16	6028	3.00	3.00	3.00
17	3946	4.00	4.00	5.00
18	2774	4.00	3.33	3.33
19	775	5.13	3.79	3.48
20	11A	4.50	3.00	3.50
21	2913	3.91	2.94	3.53
22	3467	5.20	3.40	3.60
23	5946	5.00	4.00	3.00
24	461	3.88	2.94	2.56
25	2520	6.00	4.00	4.00

1.4 Plant Protection Studies

1.4.1 Efforts for Maintaining Contamination Free Tissue Culture Lab and Nursery

Contamination refers to the growth or existence of unwanted microorganisms or other materials in cultures. It is a major threat in tissue culture causing both economical and effort loss. To avoid this, aseptic conditions should be maintained in the laboratory. Microorganisms including bacteria, fungi, viruses, mycoplasma etc are the common causes of contamination in plant tissue cultures. The common procedures to reduce contaminating organisms are:-

- Selection of healthy mother plants for propagation
- Surface sterilization of explants
- Sterilization of media, glass wares and other devices used for tissue culture

procedure
- ❖ Inoculation in sterile atmosphere - sterile laminar air flow chambers
- ❖ Maintaining aseptic culture rooms

Any defects in the above parameters may lead to contaminations. Determining the source of contamination is an effective way to eliminate it.

1.4.1.1. Identification of Tissue Culture Contaminations

The detection of contaminants incorporates the study of its cultural, physiological and biochemical properties. Such an effort includes indexing of cultures, followed by identification and characterization of contaminant through various macroscopic and microscopic studies.

1.4.1.1.1 Identification of fungal contaminations in plant tissue culture laboratory

Fungi are unicellular or multi cellular organisms which live either as saprophytes or parasites. They are the major contaminating organisms in the tissue cultures because of their simple and rapid reproductive processes through asexual and sexual spores. Elimination of fungal contaminants is crucial for the successful tissue culture production, as the fungi species during their rapid growth, utilize the culture media and destroy the explants.

Lacto phenol cotton blue of tear mount staining technique was employed for fungal identification. A drop of Lacto Phenol Cotton Blue (LPCB) stain was placed on the centre of a clean glass slide. Using a flame sterilized needle a few fungal mycelium was placed on the stain and the mycelia was gently teased and spread using a sterile needle. Fungal smear was covered with a cover slip and observed under 40X microscope after 30 seconds. Various fungal species were identified based on their morphological characteristics from the banana and pineapple tissue culture bottles.

Table 28. Fungal Contaminations in banana tissue culture media

Month of occurring	Plant Variety along with media	Type of contaminants	% of Occurrence
May- 2012	N BA_2[1]	*Fusarium spp.*	42.85%
	n BA_2	*Yeast spp.*	14.28%
		Cladosporium spp.	28.57
		Sporotrichosis spp.	14.28%
June - 2012	n BA_2	*Phythophthora spp.*	33.33%
		Diplococci spp.	16.66%
		Fusarium spp.	50%
July - 2012	n BA_1[2]	*Fusarium spp.*	33.33%
		Yeast spp.	66.66%

[1] Sub culturing media for Nendran inflorescence
[2] Fresh inoculation media for Nendran inflorescence

October -2012	NBA_2 B BA_2 n BA_2	*Aspergillus spp.* *Fusarium spp.* *Pencilium spp.*	25% 25% 50%
November- 2012	n BA_2 NBA_2 P BA_2[3]	*Aspegillusspp.* *Fusarium spp.* *Cladosporiumspp.*	12.5% 12.5% 12.5%
	n BA_2	*Mucor spp.* *Yeast spp.*	25% 37.5%
December - 2012	n BA_2	*Mucor spp.* *Fusarium spp.* *Yeast spp.*	14.28% 4.76% 38.09%
	NBA_2	*Pencilium spp.*	28.57%
January- 2013	NBA_2 n BA_2	*Phythophthora spp.* *Yeast spp.*	40% 60%

Table 29. Fungal and bacterial Contaminants and their percentage of occurrence in Pineapple tissue culture media

Month of occurring	Media	Type of contaminant	% of contamination
October 2012	MD2 PA_2[4] MD2 IN MD2 PA_2 MD2 PA_2	*Cladosporium spp.* *Micrococcus spp.* *Pencilium spp.* *Fusarium spp.*	16.66% 33.3% 16.66% 33.3%
November 2012	MD2 PA_2 MD2 PA_2 MD2 PA_2	*Fusarium spp.* *Aspergillus spp.* *Pencilium spp.*	50% 25% 25%
December 2012	MD2 PA_2 MD2 PA_2 K IN K PA_2[5]	*Pencilium spp.* *Aspergilus spp.* G+ve bacilli G+ve cocci	20% 20% 40% 20%

[3] Poovan subculture media
[4] Subculture media for MD2 Pineapple
[5] Subculture media for Kew pineapple

Table 30. Identification of Contaminants in Nendran Fresh Inoculants

SI No	Sample No	Macroscopy	Microscopy
1	n[6] 63, n 69	White creamy colonies	Gram positive round yeast cells
2	n 56, n 103, n 135, n 126, n 136, n 145, n 139, n 144, n 88, n 100	White cottony growth on the explants	*Fusarium spp.*
3	n 64, n 117, n 58	White mucoid colonies	Gram positive rods
4	n 62, n 123, n 125, n 127, n 134, n 116, n 81, n 92, n 65, n 133, n 132	White creamy mucoid growth	Gram Negative rods
5	n 74,n 118, n 97, n 106	White mucoid growth	Gram positive cocci

Table 31. Observations of Lacto phenol Cotton Blue Staining

Sl.No.	Macroscopy	Microscopy	Staining method used	Inference
1.	Green coloured colonies with white margins	Broom shaped conidiophores	LPCB	*Pencilium spp*
2.	White cottony growth on media	Sickle shape spores at the tip	LPCB	*Fusarium spp. spp.*
3.	Dark bluish green colonies	Conidia spores arise from a foot cell	LPCB	*Aspergillus spp*
4.	Grey/creamy hair like mycelia growth	Non septate mycelia	LPCB	*Mucor spp.*
5.	White creamy colonies	Gram positive large round cells	Gram staining	*Yeast spp.*
6.	Creamy hair like growth	Septate mycelia, Rhizoids were found	LPCB	*Rhizopus spp.*

[6] Fresh inoculation media for Nendran inflorescence

A wide range of microorganisms cause contamination in tissue culture laboratory. Fungi, yeast, molds and bacteria were the predominant microbes. Among them fungi were the major contaminants, 73.13% of consisting of fungal contamination and of bacteria were 26.87%.

Figure 1 a. Macroscopic observation of *Aspergillus spp.*

Figure 1.b. Microscopic Observation of *Aspergillus spp.*

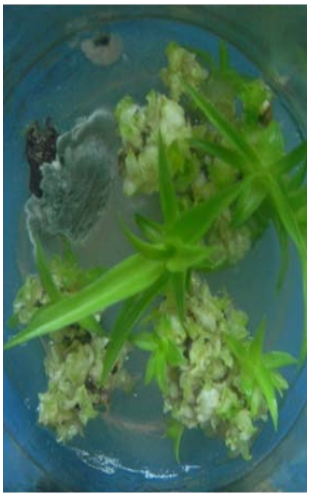


Figure 2 a. Macroscopic observation of *Pencilium spp.*

Figure 2 b. Microscopic observation of *Pencilium spp.*

Figure 3 a. Macroscopic observatio of Yeast

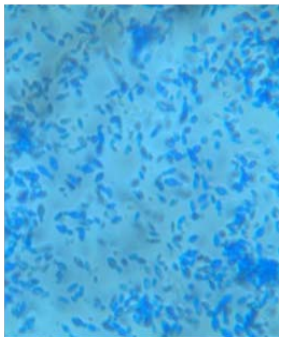


Figure 3 b. Microscopic observation of Yeast

Figure 4 a. Macroscopic observatior *Fusarium spp.*

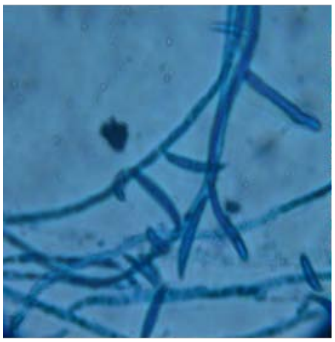


Figure 4 b. Microscopic observation of *Fusarium spp.*

Figure 5 a. Macroscopic observation of *Mucor spp.*

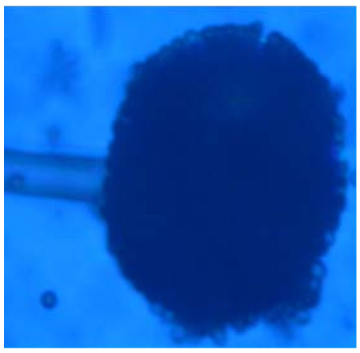


Figure 5 b. Microscopic observation of *Mucor spp.*

Figure 6 a. Macroscopic observation of *Mucor spp.*

Figure 6 b. Microscopic observation of *Mucor spp.*

Figure 27. Macroscopic and Microscopic observations of various fungi

1.4.1.1.2 Identification of bacterial contaminations in plant tissue culture laboratory

Bacterial contamination can cause severe losses to micro propagated plants at each stage of growth. The contaminated plants may show no visible symptoms, exhibit reduced multiplication and rooting rate, or they may die. Even when there is no visible symptom in vitro the contaminant may become pathogenic on transfer of plants to greenhouse or field. Therefore, preventing or avoiding contamination is critical to successful micro propagation. The classical approach to bacterial identification involves preliminary microscopic examination of the Gram stained preparations for its categorization into two broad groups (Gram positives and Gram negatives) which would later form the basis for the selection of biochemical tests to be performed.

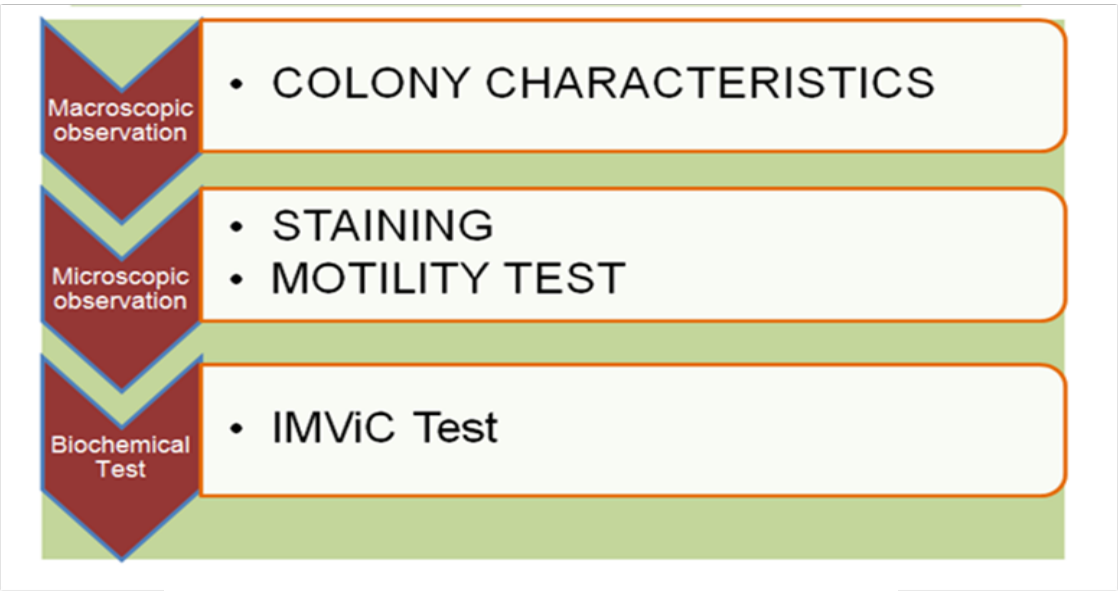


Figure 28. Steps Involved in bacterial identification

Gram Staining of Bacteria

In this staining technique bacterial smear is subjected to 4 different stains i.e.; Crystal violet (primary color), Iodine solution (mordent), alcohol (decolorizing solution) and safranin (counter stain). The bacteria which retain the primary stain (appear dark blue/violet) are called Gram positives, whereas those that lose the primary stain and counter stained by safranin (appear pink/red) are referred as Gram negatives.

Take a clean glass slide and prepare a thin smear of bacterial culture, and heat fixed. Placed few drops of Crystal violet for 60 seconds and washed the slides with distilled water. Covered the smear with iodine solution for 60 second and washed off with 95% ethyl alcohol drop by drop until no more color flows from the smear. Washed the slide with distilled water and applied safranin (counter stain) for 30 seconds then washed the slide with distilled water and air dried. Observed the slide under oil immersion microscope. Microscopic examination reveals the morphology and arrangement of the cells.

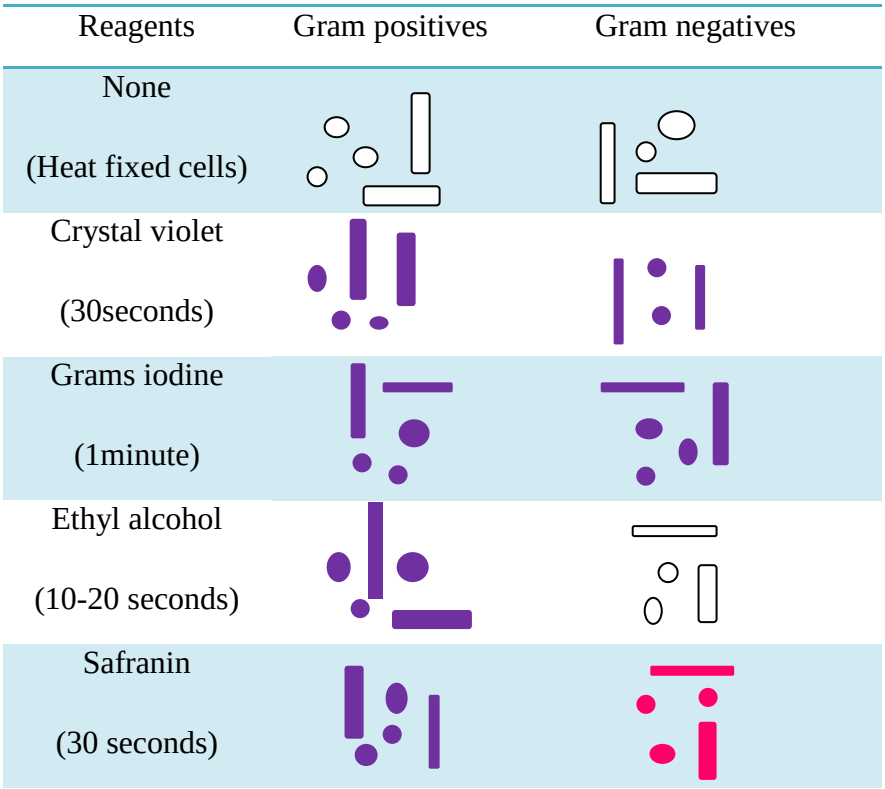


Figure 29. Gram staining reaction of Gram positives & Gram negatives

Precautions to be taken for a sterile tissue culture lab

- Regular cleaning of Tissue culture Lab using Dettol/Lysol
- Give more attention in media preparation when measuring, mixing, pouring, sterilization and transfer to Tissue Culture laminar air flow
- Media for culturing as well as sub culturing should be sterilized in autoclave, and once sterilized should not be again sterilized; it will change the composition of ingredients.
- The room should have a double door and it should be kept closed at all times
- All culture vessels including pipettes should be sterilized by dry heat in Hot Air Oven at 160-180^{O}C
- Staff should wear apron/lab coat, gloves, mask (on head and mouth) while working inside Tissue culture lab
- Tie hair properly and wear minimum ornaments and accessories. Cut the nails regularly and don't use nail polishes.
- Used glass wares and media must be decontaminated by autoclaving before washing and disposal.

- The platform of LAF and hands of staff working must be wiped with 70%alcohol
- Routine close observation of tissue culture flask and bottles are necessary (examine the presence of white/cream colored spreading or any colored mycelia growth)
- Remove all infected flasks immediately seeing any kind of contamination
- Lab coat, gloves and mask must be autoclaved after washing & drying
- Before all tissue culture operation the laminar air flow should be pre-sterilized by putting U-V light for 45min.The platform must be wiped with spirit or 70% alcohol every corner
- Spray spirit in LAF before and after the work
- Media preparation room, inoculation and culture maintenance room should be fumigated with formalin at regular intervals. All the doors, ventilations, electric circuits should be off before fumigation
- Keep maximum cleanliness by each and every worker

1.4.1.3. Identification of Contaminations in Nursery

1.4.1.3.1 Potting Mixture Analysis of Passion Fruit Roof Top Nursery

Objective

To identify the infective agent on passion fruit seedlings and potting mixture

Technical Programme

16 potting mixture samples were collected from the roof top nursery and weighed 1g of samples each and suspended to 9ml sterile distilled water tubes. Then directly plated the sample to Sabouraud Dextrose Agar (SDA) plates and marked each plate. Incubated the plates at room temperature for 3 days. After proper incubation stained the colonies using LPCB staining method.

Observation & Result

Macroscopic: Grey/white color hair like growth on SDA

Microscopic: Well developed, highly branched mycelium, Coenocytic hyphae without septa, Rhizoids were present and small round spores

The fungi isolated from the samples were Rhizopus Spp.

Inference

Fungi Rhizopus is saprophytic and nonpathogenic hence no fungicides needed.

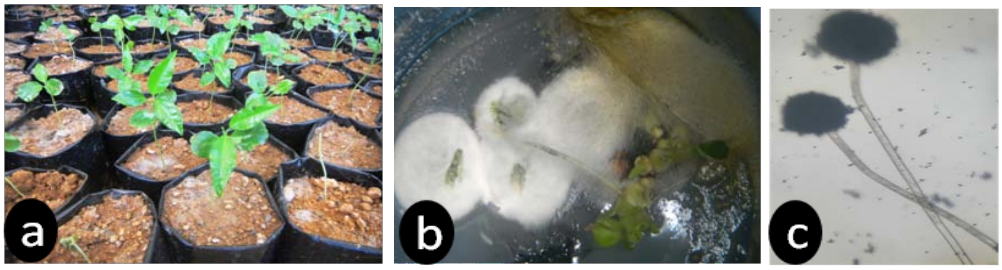


Figure 30. (a) Roof top soil samples (b) *Rhizopus spp.* on SDA (c) *Rhizopus spp.* (40X)

1.4.1.3.2 Identification of fungal pathogen in soil samples through serial dilution technique

Objective

To identify the pathogenic fungi present in soil of passion fruit nursery

Requirements

Soil sample, SDA plates and routine microbiology laboratory equipments

Technical programme 1

5 soil samples were collected from the roof top nursery. Weighed 1g of soil samples and suspended in 9 ml sterile distilled water and directly spread the sample to SDA plates. Plates were incubated at room temperature for 3 days and stained the colonies developed using LPCB staining method.

Observation and Result

Table 32. Observations of Lacto phenol Cotton Blue Staining

Sample No.	*Macroscopic*	*Microscopic*
S1	White cottony colony, Green color colony with white margins	*Fusarium spp.* *Pencilium spp.*
S2	White cottony colonies, Black powdery colonies, Light bluish colonies	*Fusarium spp.* *Aspergillus spp.* *Pencilium spp.*
S3	Yellow color thick mycelia growth Dark bluish green colonies Grey white fluffy colonies	*Aspergillus spp.* *Aspergillus spp.* *Phytophthora spp.*
S4	White cottony colonies Cream color mucoid colonies Bluish color colonies	*Fusarium spp.* *Yeast spp.* *Pencilium spp.*
S5	Cream color colonies White cottony colonies Light bluish colonies	*Yeast spp.* *Fusarium spp.* *Pencilium spp.*

NB: S1- S5: Soil samples

Direct plating of soil suspension showed numerous fungal colonies and they constitute a major place among the soil micro flora. Most of them are pathogenic to plants and hence it's important to know the population density of these fungi in the soil. The soil borne fungi isolated and their total population enumerated using serial dilution method.

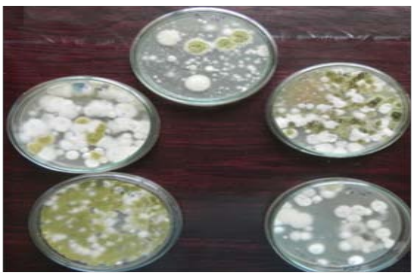


Figure 31. Different fungal colonies on SDA

Technical Programme 2

5 different soil samples were collected from nursery plant pot. Weighed 1g of soil samples and transferred to the test tube containing 9ml sterile water (dilution -10^{1}). Arranged 5 sets of test tubes, each set contained 9 ml of sterile distilled water. Shaked and homogenized the first tube and transferred 1ml from it to the second. Similarly, 1ml sample was serially transferred from 10^{-2} dilution into third tube containing 9ml of sterile water to get a final dilution of 10^{-3}. Repeated the procedure for 10^{-4}, 10^{-5}, 10^{-6} dilutions. The same procedure was followed for remaining four soil samples. Aseptically poured 1ml soil suspension from 10-1, 10-3, and 10-5 into respective SDA plates (incorporated with antibiotic penicillin 30mg /liter). Plate was gently rotated to spread the suspension on medium. The plates were incubated at room temperature for 4 days. Colony was sub-cultured and characterized

Table 33. Observations of Lacto phenol Cotton Blue Staining

Sample dilution taken for plating	No. of colonies	Macroscopy	Identification
S1 10^{-1}	TNTC[7]	Large white cottony colonies Light green colonies	*Fusarium spp.* *Pencilium spp.*
S2 10^{-1}	TNTC	Large white cottony colonies Black powdery colonies	*Fusarium spp.*
S3 10^{-1}	TNTC	Greenish white color colonies	*Pencilium spp.*
S4 10^{-1}	TNTC	Large white cottony colonies Bluish green colonies	*Fusarium spp.* *Aspergillus spp.*
S5 10^{-1}	TNTC	White colonies Cream color colonies	*Fusarium spp.* *Yeast spp.*

[7] Too numerous to count

S1 10^{-3}	05	Dark green colonies	*Aspergillus spp.*
	13	Light green colonies	*Pencilium spp.*
	09	White cottony growth	*Fusarium spp.*
S2 10^{-3}	14	Greenish white color colonies	*Pencilium spp.*
	13	White cottony growth	*Fusarium spp.*
S3 10^{-3}	18	White cottony growth	*Fusarium spp.*
S4 10^{-3}	21	White cottony growth	*Fusarium spp.*
	11	Cream color colonies	*Yeast spp.*
S5 10^{-3}	12	Cream color colonies	*Yeast spp.*
	08	Black powdery colonies	*Rhizopus spp.*
S1 10^{-5}	2	Dark blue/grey color colonies	*Aspergillus spp.*
S2 10^{-5}	3	White color colonies	*Fusarium spp.*
S3 10^{-5}	5	White large cottony growth	*Fusarium spp.*
S4 10^{-5}	1	Large white cottony colonies	*Fusarium spp.*
S5 10^{-5}	4	Large white cottony colonies	*Fusarium spp.*

S1, S2, S3, S4, & S5: Soil samples

Figure 32. Soil sample taken for serial dilution

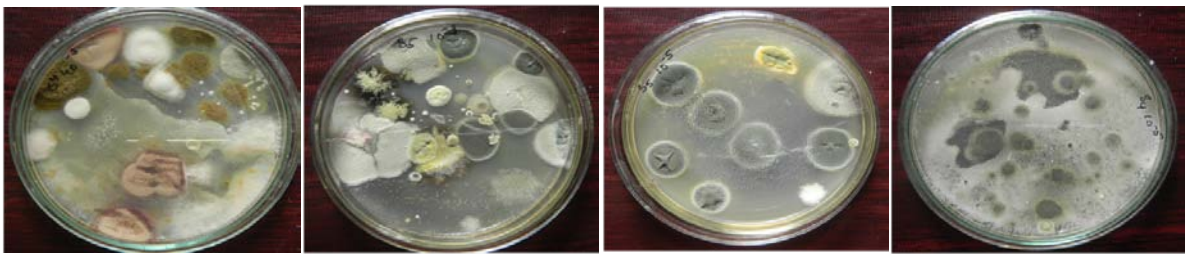
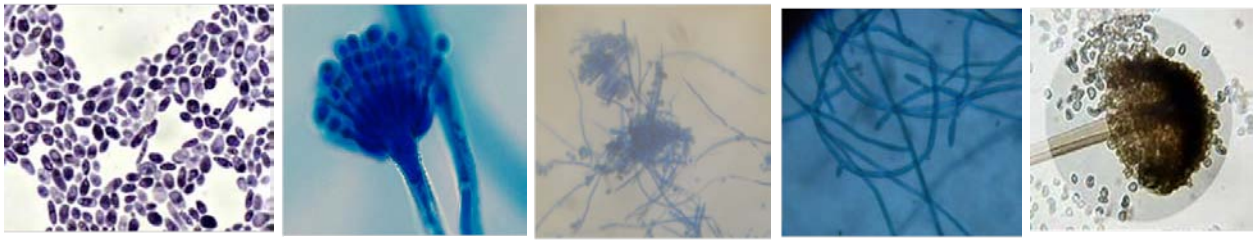


Figure 33. Different fungal colonies on SDA plates



Yeast spp *Aspergillus spp* *Pencilium spp* *Fusarium spp* *Rhizopus spp*

Figure 34. Observations of Lacto phenol Cotton Blue Staining

Result

Antifungal sensitivity tests were done against Pencilium, Aspergillus, Yeast, and Fusarium. Hexaconazole, Indofil, SAAF, Bavistin trials were done. 0.3% (3g/1000ml) Bavistin was recommended to spray on soil and leaves. *Fusarium spp*. was found to be in large number from 5 soil samples studied. This may be the reason for wilt in passion fruits planted in rooftop nursery.

1.4.2 Identification of Pathogens from Diseased Plants

1.4.2.1 Isolation and identification of pathogen for white leaf spot disease in Pineapple

Objective

To isolate and identify the causative organism of white leaf spot disease in pineapple leaf.

Requirements

SDA & Nutrient Agar (NA) plates, Sterile distilled water, test tube, mortar and pestle, routine lab equipments

Technical Programme

Infected plants were observed and symptoms of the disease were noticed. Diseased leaf samples were collected randomly from different areas of the pineapple field. Soil samples were also collected both from the rhizosphere region (The region of plant root and soil) of infected plants and uninfected plants which were very few in number.

Microbiological Analysis of diseased leaf samples

1. Serial dilution of infected leaf sample

1g of leaf sample was aseptically weighed and the sample was ground using a sterile mortar and pestle. 9ml of sterile distilled water was added and serial diluted the sample up to 10^{-5} dilution. 0.1ml of the sample from 10^{-1}, 10^{-2}, and 10^{-5} dilutions were plated to nutrient agar and SDA. The plates were incubated at room temperature for 4-5 days. They were daily observed for the growth of suspected fungi.

2. Direct analysis of infected leaf sample

Diseased portion of the leaf was aseptically cut with a sterile scissor and directly inoculated into nutrient agar and SDA media. Incubated the plates at room temperature for 4-5 days.

3. Microbiological analysis of soil samples

1g of soil sample was weighed and aseptically transferred to 9ml sterile distilled water and serial diluted the sample up to 10^{-5} dilution. 0.1ml of the sample from 10^{-2} and 10^{-3} dilutions were plated to sterile SDA plates for fungal growth and 0.1ml of the sample from 10^{-4} and 10^{-5} dilutions were plated to sterile nutrient agar plates for bacterial colonies. Incubated the SDA plates at room temperature for 3 days and NA plates at 37°C for 24 hour.

Observation and Results

Symptoms on leaf: White patches on the leaves. Initially it is seen as a white spot only then it develops and spread throughout the leaf as white powdery appearance.

After 5 days of incubation fungal colonies developed on SDA plates. The predominant fungal colonies developed on the plates were subjected to LPCB staining to get the microscopic appearance of those fungi

Macroscopic character: Brown centered colony with white margins

Microscopic character: Septate hyphae with conidial spore, Conidia are cylindrical in shape, Conidia are terminal each conidiophore contains a single conidium.

On the basis of macroscopic and microscopic morphology the causative organism of White spot disease of pineapple leaf was by *Cercospora spp.* spp.

Cercospora spp. was subcultured on SDA plates for further studies and analysis

Figure 35. White leaf spot of Pineapple

Figure 36. Macroscopic view of *Cercospora spp.*

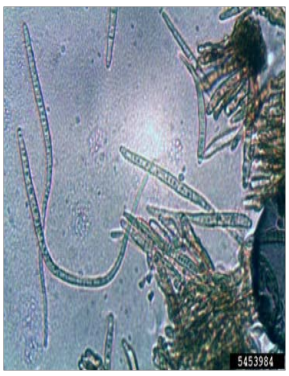


Figure 37. Microscopic view of *Cercospora spp.(40X)*

1.4.3 Bacteriological Analysis of Water Sample from Tissue Culture Lab

Objective

To analyze whether the water sample is having coliforms or not

Requirements

Water sample, Lactose broth, Nutrient agar plates, Culture tubes, and Durham's tube, Bunsen burner, sterile pipette, incubator, glass marker, and Bunsen burner

Technical Programme

The water sample was insufficient to do the complete Most Probable Number (MPN) test so 0.1ml of water sample was directly inoculated to lactose broth and Eosin Methylene Blue (EMB) agar. The plate and tubes were incubated at 37°C for 48 hours. For conformation Green metallic

sheen colonies on the EMB plate were inoculated into a fresh nutrient broth and were incubated at 37°C for 24 hours. Carried out Gram staining and motility from the broth culture.

Observation

Lactose broth showed acid and gas production. The sugar lactose was fermented by the bacteria results in the production of acid which is indicated by the phenol red indicator in the medium. This turns the color of the medium from red to yellow. Production of gas can be visible in the Durham's tube. These two indications showed a positive lactose fermentation reaction and the bacteria probably were Enterobacteriaceae spp. On EMB agar, the colonies showed green metallic sheen appearance. EMB is an indicator as well as selective media, which inhibit the growth of Gram positives. E.coli showed the metallic sheen appearance on EMB agar.

Gram's staining and hanging drop method showed that the bacteria as Gram Negative motile bacilli

Result

All the positive results showed the presence of both *Enterobacter aerogens* and *Escherichia coli* in the given water sample.

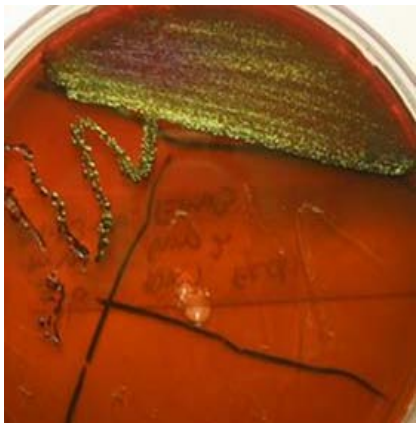


Figure 38. *E. coli* colonies on EMB agar plate

Figure 39. Control

Figure 40. Positive lactose broth

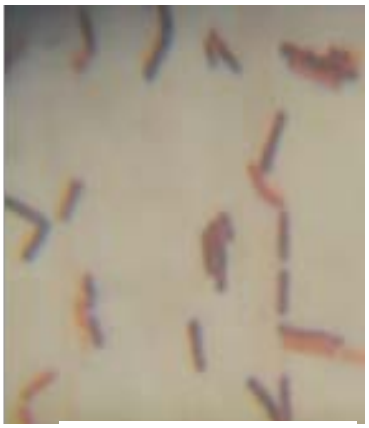


Figure 41. Gram negative rod

1.4.4 Antifungal Sensitivity Tests

1.4.4.1 Response of the fungi *Cercospora spp.* to different fungicides with varying concentrations

Objective

To determine the response of the fungi *Cercospora spp.* isolated from pineapple leaf showing white spot to various fungicides and to know about the most effective fungicides and its concentration.

Requirements

Indofil, SAAF, Contaf, Bavistin, SD broth, SDA plates, Cotton swab, test tubes and routine microbiology laboratory equipments

Technical Programme

Fungal culture was inoculated into Sabourauds Dextrose broth and incubated at room temperature for 2-3 days.

Different concentrations of the following fungicides were made

Table 34. Concentrations of various fungicides used in antifungal sensitivity assay

Fungicides used	Concentrations used		
Indofil	0.15%	0.3%	0.6%
SAAF	0.1%	0.2%	0.4%
Contaf	0.1%	0.2%	0.4%
Bavistin	0.1%	0.2%	0.4%

All fungicides were made as 10ml stock solution. Fungal culture from SD broth was swabbed on an SDA plates. Plates were dried for 5 minutes. 3 wells were cut on the plate and 0.1 ml of the fungicides of different concentrations was added to the respective wells. Plates were incubated at room temperature for 5 days. After incubation the zone of inhibition was noted for each fungicide.

Results and Discussions

Table 35. Response of *Cercospora spp.* spp to different doses of fungicides

Fungicides used	*% used*	*Zone of inhibition*	*Inference*
Indofil	0.6%	24	++
	0.3%	13	-
	0.15%	10	-
SAAF	0.4%	28	++
	0.2%	20	++
	0.1%	12	-
Contaf	0.4%	36	+++
	0.2%	22	++
	0.1%	12	-
Bavistin	0.4%	0	-
	0.2%	0	-
	0.1%	0	-

+++ More sensitive ++ less sensitive - resistant

The fungicide Contaf showed better inhibition on *Cercospora spp.*. 0.4% was more effective against the fungi *Cercospora spp.*. 0.6% Indofil, 0.4% & 0.2% SAAF also showed an average inhibitory effect on the fungi. *Cercospora spp.* was resistant against the fungicide Bavistin.

Zone of inhibition by different concentrations of Indofil

Zone of inhibition by different concentrations of Contaf

Zone of inhibition by different concentrations of Bavistin

Figure 42. Response of fungi *Cercospora spp.* to various fungicides

1.4.5 Effect of Copper sulphate ($CuSO_4.5H_2O$) on *Candida spp.* isolated from the rotted pineapple sample

Objective

To determine the effect of $CuSO_4.5H_2O$ on *Candida spp.*

Principle

Candida spp. is yeast like fungi show white cream color large round colonies on SDA and PDA. LPCB staining shows that large ovoid, single or budding yeast like appearance. Fresh inoculum shows pseudo mycelial characteristics. Copper sulphate is a fungicide which inhibits the growth of fungi. Different concentrations of copper sulphate were (3%, 3.5%, 4% and, 4.5%) tried against *Candida* to find out the most effective one.

Technical Programme

SDA plates were prepared and made the lawn culture of *Candida* spp. on the plates. Prepared 3%, 3.5%, 4% and 4.5% of copper sulphate solutions. Added 1ml of the above copper sulphate concentration solutions to appropriate plates. Incubated the plates at room temperature for 3-4 days.

Observations

Table 36. Response of Candida *spp.* to different doses of $CuSO_4.5H_2O$

Concentrations of $CuSO_4.5H_2O$ used	*Zone of inhibition*	*Inference*
3%	24mm	***
3.5%	23mm	***
4%	20mm	**
4.5%	19mm	**

*** Good ** Average

Result and Discussion

From the above observations $CuSO_4.5H_2O$ with 3% and 3.5% concentrations were found to be most effective against *Candida* spp.

Figure 43. *Candida* spp. on SDA

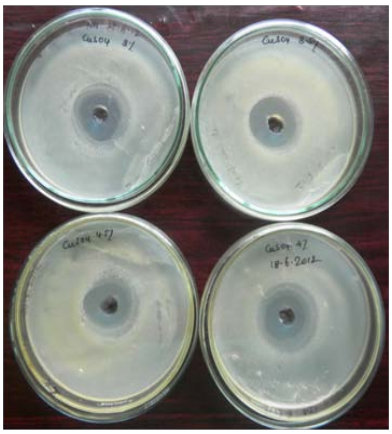


Figure 44. Zone of inhibition

1.4.6 Plant Health Clinic Releases

Plant Health Clinic - Release 4: Passion fruit wilt by Fusarium ?

Crop & variety: Passion fruit Yellow

Symptoms: Leaf colour changed to pale green and further gradation to yellow leading to the leaf wilt. Defoliation of leaves. Necrotic girdling of the plant collar and die back. Infected plants showed the presence of *Fusarium spp. spp.* spores and hyphae on Lacto phenol Cotton Blue Staining (LPCB).

Location: Experimental farm of Pineapple Research Station at NAPCL, Nadukkara, Muvattupuzha, Ernakulum District.

Period: November 2012 - February 2013

No. of plants affected: 4 plants (88Y-2, 88Y-3 & 45Y-4, 51Y-2)

Control measures taken: Applied Indofil 3g/l and then Bavistin , 3g/l after 1 month. Once affected the plant is gone.

Figure 45. Fusarium Wilt of Passion fruit

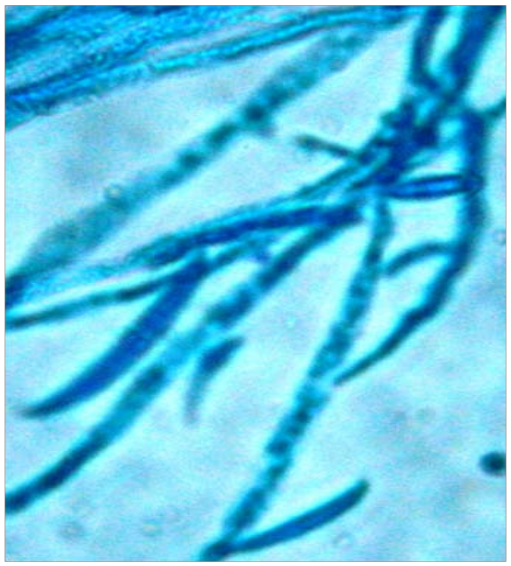


Figure 46. Fusarium spores and hyphae

Plant Health Clinic - Release 5: Root rot of passion fruit by Phytophthora ?

Crop & variety: Passion fruit Yellow

Symptoms: Phytophthora root and crown rot disease affected both field as well as nursery plants. Mild cholorosis followed by wilting, defoliation and death. There is a change in leaf colour from pale green, to colourless with leaves reaching a light copper colour. Infected plants showed the presence of hyphae and spores of phytophthora on Lacto phenol Cotton Blue Staining (LPCB).

Location: Experimental farm of Pineapple Research Station at NAPCL, Nadukkara, Muvattupuzha, Ernakulum District.

Period: December 2012 - February 2013

No. of plants affected: 3 plants (86Y-2, 86Y-1, 66Y-1)

Control measures taken: Drenched SAAF, 2.5g/l and then 2.5g/ l Indofil 1 month after. Once affected the plant is gone.

Figure 47. Root & collar rot

Figure 48. Diseased plant leaf

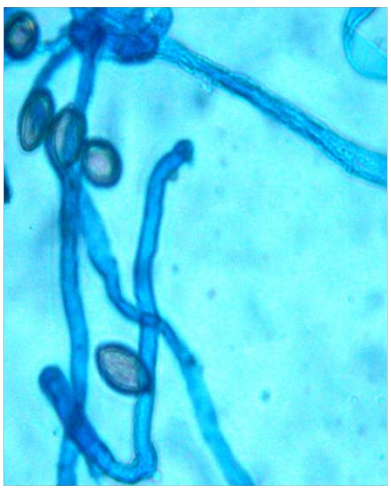


Figure 49. Phytophthora species

1.4.7 Diagnostic Team visits

Table 37. Diagnostic Team Visits

Date	Diagnostic Team visits
03/05/2012	Pattambi Diagnostic Team led by Dr. Raji P., Asso. Professor of Plant Pathology, RARS, Pattambi. visited the station and inspected the field for diagnosing various pest and disease problems in pineapple and passion fruit.
22/05/2012	KAU Diagnostic Team led by Dr. Jim Thomas, Professor (Entomology) and Head, Directorate of Extension, Mannuthy & Dr. Anitha Cheriyan. K, Professor (Plant Pathology) BRS, Kannara, visited the station and inspected the pineapple and passion fruit fields at NAPF, Nadukkara and other private pineapple plantations in the visits such as that of Mr. Vincent Varghese, Nambiaparambil, Mullapuzhachal, Kaliyar estate etc. and collected plant samples for further studies. The team identified the incidents of mealy bug, scale insects, heart rot etc. as the greatest problems in pineapple plantations. The KAU diagnostic team also submitted the report to the University.

Figure 50. Diagnostic Team visits: (a) Dr. Jim Thomas, Professor (Entomology) and Head, Directorate of Extension & Dr. Anitha Cheriyan. K, Professor (Plant Pathology) BRS, Kannara inspecting PRS field (b) & (c) Mealy bug infested roots (d) Dr. Raji P., Asso. Professor of Plant Pathology, RARS, Pattambi investigating field pathogens

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.

Figure 51. (a) Passion Fruit- Purple (b) Passion Fruit – purple in vines (c) Passion Fruit – Yellow (d) Passion Fruit – Yellow in vines

2.1 Study on germination and seed viability of passion fruit seedlings

Objective

To evaluate the percentage of seed viability and germination in different passion fruit varieties

Technical Programme

50 seeds each of 5 different varieties namely 35-Yellow, 86-Yellow, 36-Purple, 34-Purple and Vazhakulam-Purple were taken. Seeds were soaked separately in distilled lukewarm water for 18 hours in glass beakers. Equal quantity (4ml) of water was used for each variety of seeds. Seeds were carefully stirred 3-4 times, regularly at an interval of 7-8 hours. Beakers were labeled carefully.

Soil for seed sowing was prepared my mixing 10 Kg solarised soil + 1 Kg Cow dung +100g Trichoderma + 100g Neem Cake. The soil was thoroughly mixed and irrigated well. Soil was taken in plastic trays. 50 seeds of each variety was sowed separately in 5 different plastic trays and labeled. Trays were kept in mist chamber and irrigated regularly. Seeds were observed on a daily basis to identify any disease incidence.

Results and Discussions

Germination of passion fruit seeds started with in the first week of sowing. Rate of germination was slow the first week, which was slightly increased in the second week and third weeks. By the fourth week the total germination was more than fifty percentage for each variety. Germination was again slowed by the fourth week. General conclusion that can be obtained from

this study is the time of germination and general trend of germination. Average time required for the germination of passion fruit seeds is one month with highest percentage of seed germination in the third week. In between the varieties used for study here 86-Yellow and Vazhakulam – Purple showed 78 and 72 percentage of germination respectively. Least percentage of germination was showed by 34-Purple variety.

Table 38. Total number of passion fruit seeds germinated over a period of four weeks

Variety	seeds sowed	First week		Second week		Third week		Fourth Week	
		No. of Seeds Sprouted	Viability (%)	No. of Seeds Sprouted	Viability (%)	No. of Seeds Sprouted	Viability (%)	No. of Seeds Sprouted	Viability (%)
35- Y	50	3	6	10	20	13	26	8	16
86- Y	50	2	4	2	4	20	40	15	30
36- P	50	3	6	5	10	14	28	10	20
34 -P	50	2	4	5	10	13	26	9	18
V- P	50	3	6	3	6	18	36	12	24

Table 39. Total number of passion fruit seeds germinated and viability percentage over a period of four weeks

Variety	No. of Seeds sowed	Total Seeds Germinated	Total Viability (%)
35-Yellow	50	34	68
86-Yellow	50	39	78
36- Purple	50	32	64
34 -Purple	50	29	58
V- Purple	50	36	72

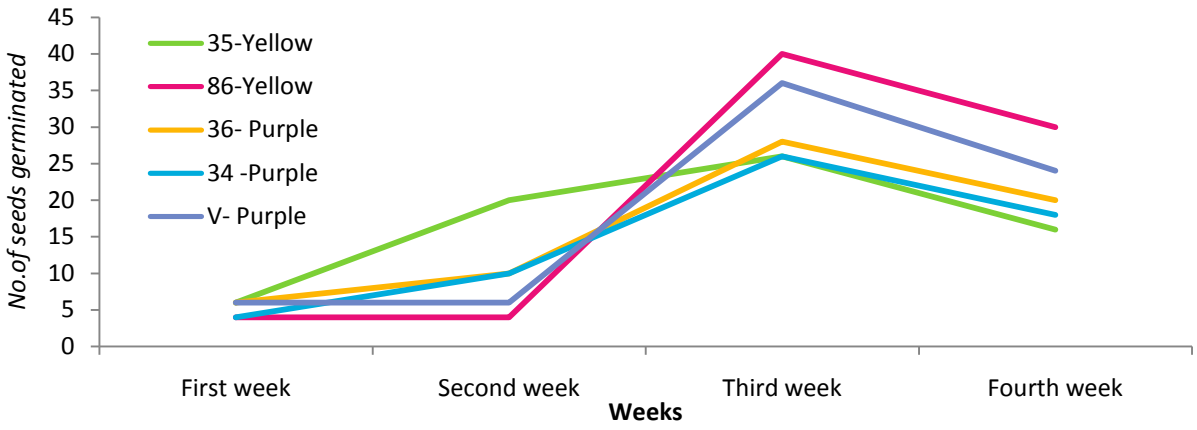


Figure 52. Viability percentage for a period of four weeks

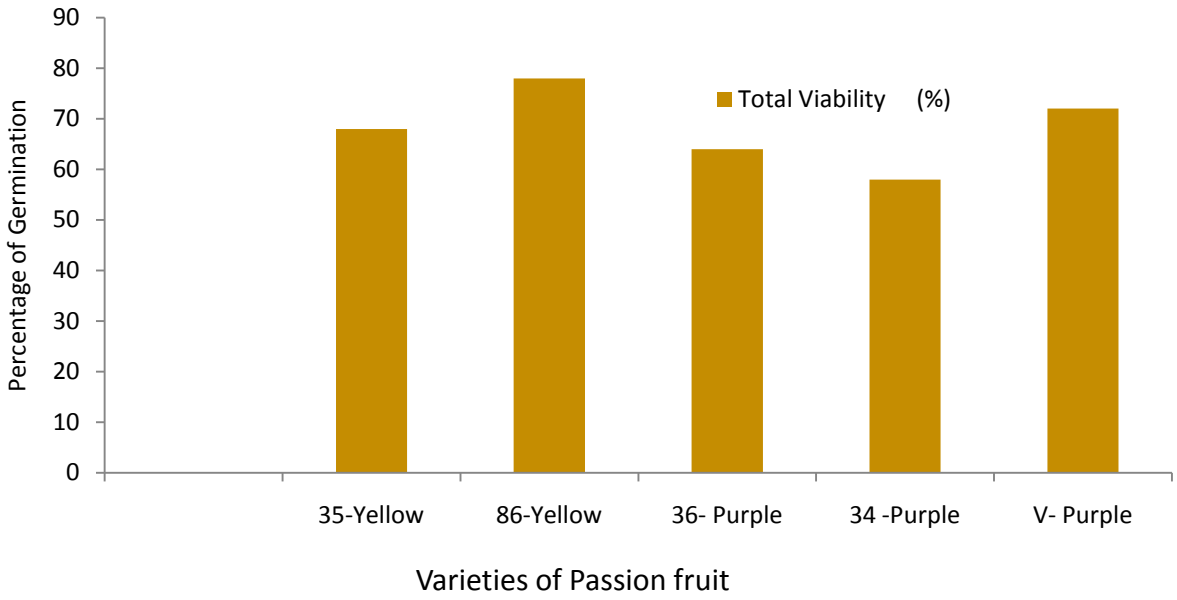


Figure 53. Percentage of germination of different varieties of passion fruit seeds

Figure 54. Germinated seedlings of different varieties of passion fruit seedlings

2.2 Study on the effect of nodal length on rooting of purple passion fruit stem cuttings

Passion fruit is a cross pollinating species and so propagation of these plants through seed germination can affect the purity of the germplasm. Vegetative propagation through the production of rooted stem cuttings can be effectively used for production of large number of propagation materials. Factors affecting the rooting of stem cuttings are moisture or humidify, temperature, aeration, and length of nodal cuttings, age of vine and various treatments on the stem cuttings kept for rooting.

Objective

This study objectives to identify the appropriate nodal length for effective rooting of stem cuttings.

Technical programme

Passion fruit vines of same age were collected from locally available farmers. Vines were cut into pieces of different nodal length. A total of sixty stem cuttings were selected, which was classified into four study groups based on the length of the cuttings, as 2- node, 3-node, 4-node and 5- node. Tendrils and leaves were removed from the bottom shoot region. The branch was

diagonally cut 2 cm above the upper and 3 cm below the bottom growth point. Three fourth of each leaf was removed to avoid dehydration. Bottom region of the stem cutting was dubbed in rooting mixture prior to planting. For preparing 1000ppm solution of rooting mixture, 0.5 g of IBA (Indole -3-Butyric acid) was dissolved in 5-7 drops of NaOH. Later this solution was made up to 500 ml. Each study group contained a total of 15 stem cuttings which were all given equal treatments. Cuttings were planted in poly bags contained potting mixtures. To prepare potting mixture 10 Kg of soil was enriched with 1Kg Cow dung, 100g Neem cake and 100g Tricoderma. Soil was irrigated and kept covered over night. On the very next day poly bags were prepared with this soil. Experiment was conducted in the mist chamber to provide uniform environmental conditions like humidity, temperature and aeration. Watering of stem cuttings was done twice a day and cuttings were observed on a regular interval of three days. Cuttings which retained green color one was kept and the unhealthy ones were removed. Period of study was extended for 36 days.

Table 40. Representation of the number of stem cuttings survived over a period of 36 days.

Days		0	3	6	9	12	15	18	21	24	27	30	33	36
2-Noded	Survived cuttings	15	12	9	9	7	6	2	1	1	1	1	1	1
	Recovery Rate	100	80	60	60	47	40	13.3	6.6	6.6	6.6	6.6	6.6	6.6
3-Noded	Survived cuttings	15	15	15	12	12	11	11	11	9	8	7	7	7
	Recovery Rate	100	100	100	80	80	73.3	73.3	73.3	60	53.3	46.6	46.6	46.6
4-Noded	Survived cuttings	15	15	13	12	10	9	9	9	7	7	7	7	7
	Recovery Rate	100	100	87	80	67	60	60	60	47	46.6	46.6	46.6	46.6
5-Noded	Survived cuttings	15	15	12	10	9	7	6	6	3	3	3	3	3
	Recovery Rate	100	100	80	66.6	60	46.6	40	40	20	20	20	20	20

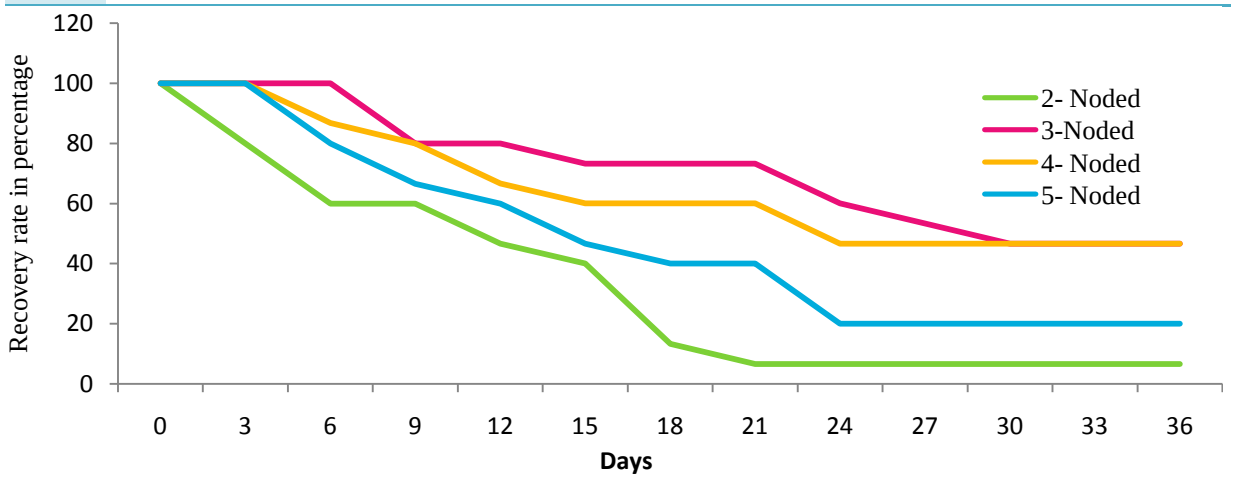


Figure 55. Recovery of stem cuttings kept for rooting over 36 days

Inference

During the first week of experiment, each study group showed a sharp decline in the number of cuttings. 3-noded and 4-noded stem cuttings showed a better survival during first week followed by 5-noded and 2-noded cuttings. Second week also demonstrated a reduction in the number of stem cuttings in each group. General trend of survival was same as first week with 3-noded and 4-noded stem cuttings displaying better survival than 5-noded and 2-noded cuttings during second week. From the third week stem cuttings showed a remarkable stability in all the four study groups. By the end of third week small budding were observed in 3-noded and 4-noded stem cuttings. Survival trend was same as previous weeks in the third week too.

Budding started by the third week was increased in the fourth week. 3-noded and 4-noded cuttings showed a survival rate of around fifty percentage. In the entire study 2-noded and 5-noded cuttings showed poor survival rate. By the fourth week budding was observed in all the survived cuttings.

Conclusion

This study put light to the general trend of rooting of passion fruit stem cuttings used for vegetative propagation. First and second week is crucial for the rooting and survival of stem cuttings used for vegetative propagation. For effective propagation usage of 3-noded or 4-noded cuttings is recommended compared to 2-noded or 5-noded stem cuttings.

Figure 56. Stem cuttings kept for rooting experiments: (a) 3 noded (b) 4 noded (c) 5 noded

2.3 KSCSTE Project: Evaluation of Passion fruit types for commercial cultivation in Kerala

Objectives

The objective of the project is to identify a high yielding superior quality passion fruit variety for commercial cultivation in Kerala so as 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.

Technical programme

Over fifty passion fruit accessions collected from different areas in Kerala and South India has been conserved and evaluated at the station for the last many years and 12 superior accessions have been identified. These selected types will be further evaluated for yield, quality and pest

and disease in a replicated field trial for evolving superior variety suited for the plains of Kerala. Superior passion fruit accessions identified in previous studies will be characterized morphologically and biochemically. These accessions of passion fruit will be evaluated for their growth, yield and quality characters and a suitable yield index will be developed. The different types will be ranked according to the yield index. The fourteen promising ones will be evaluated in detail for their yield, quality and consumer acceptance. Efforts will also be made to develop Processed products from these varieties for fresh consumption.

Research Progress

This is the first year of the project and the work involved land preparation, experimental layout, pandal preparation, preparation of planting materials, planting and crop management (shading, irrigation, manuring, plant protection, training on pandal, pollination, etc)

Passion fruit is a cross pollinating fruit crop and to ensure variety specificity, rooted stem cuttings were raised from the promising varieties already available in the experimental field of pineapple research station, Vazhakulam. Three, four and five node stem cuttings were selected from healthy mature passion vines. Tendrils and leaves were removed from the bottom shoot region and the branch was diagonally cut 2cm above the upper and 3 cm below the bottom growth points. Three fourth of each leaf was removed to avoid dehydration. Stem cuttings were dubbed in 1000 ppm solution of IBA (Indole 3-Butryic acid) prior to planting in poly bags with potting mixture. (10kg of cow dung, 100 g Neemcake and 100g Trichoderma). Stem cuttings were kept in greenhouse with mist at every three hours interval. Stem cuttings started rooting in twenty to thirty days.

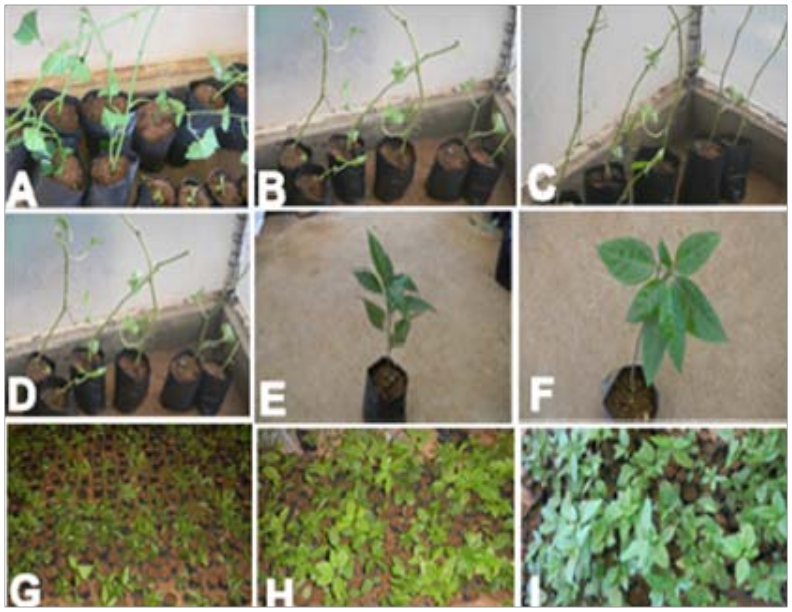


Figure 57. (A, B, C, D) Stem cuttings of passion fruits kept for rooting. (E) seedling of Yellow Passion fruit. (F) Seedling of Purple Passion fruit. (G, H, I) Passion fruit saplings ready for planting.

Seedlings were raised from the passion fruit types for which healthy rooted cuttings were not available. Seeds were sown by the end of April 2012, meant for developing seedlings for cultivation in the experimental field. Seed germination was started after 15 days and was completed by almost 30-45 days. Yellow passion fruits showed more vigorous sprouting compared to purple varieties. Growth rate of yellow variety was highest followed by purple

varieties. Passion fruit seedlings were kept in humid environment and watered regularly. Regular nursery inspection was done to identify possible pest or diseases. Seedlings having healthy appearance with 4-6 leaves and 15-20 cm length were replanted to Poly bags. Special care was given to avoid any disturbance to the taproot system of passion fruit seedlings.

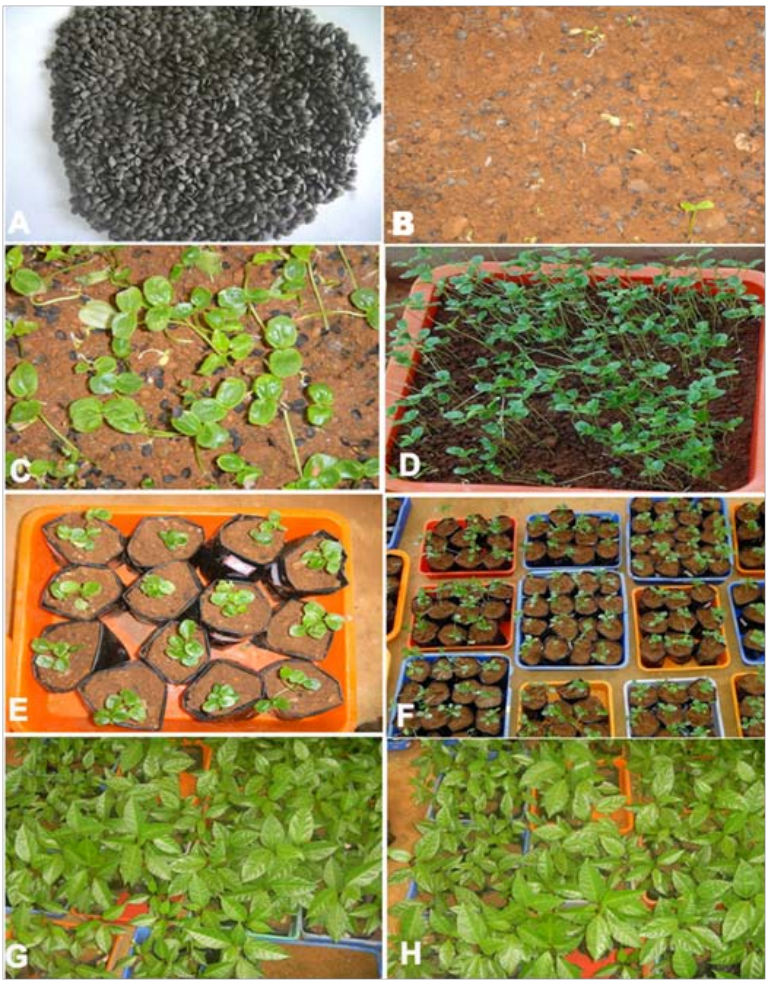


Figure 58 (A) Seeds of Passion fruit. (B) Germination of passion fruit seeds. (C) Tender seedlings of passion fruit (D) Seedlings in Plastic trays ready to be shifted to poly bags (E, F) Passion fruit seedlings replanted to the poly bags. (G, H) Passion fruit seedlings ready to be planted to the experimental plot

Plant Protection Efforts

Tender buds of passion fruits were used to be attacked by rodents which were successfully checked using pest control mechanisms. 2.5 ml/l of HILBAN was used to keep the pests away from the seedlings. As a biological control measure 5% Neem oil was mixed in 5 gm soap solution and sprayed over the seedlings.

Passion fruit seedlings showed signs of wilting. Cottony growth was seen on the soil surface. Translucent areas were seen on the tender leaves of passion fruit seedlings. Efforts taken to identify the pathogens included wet mount staining and serial dilution technique. Wet mounting

with lactophenol cotton Blue staining method confirmed the dominance of *Fusarium* spp in all samples. For a more detailed study of pathogenesis soil analysis were conducted with serial dilution techniques followed by culturing and staining procedures. Results of this study, revealed the presence of a number of fungal species namely, *Fusarium*, *Pencillium*, *Aspergillus*, *Rhizopus*, *Yeast* and *Phytophthora*. *Fusarium* spp was consistently present even after a dilution of 10^{-5}.

These results forced us to speculate *Fusarium* spp as causative agent of the wilt symptoms in passion fruits. Our assumptions were substantiated by the previous studies which reported passion fruit seedlings are slightly more susceptible to Fusarium wilt. Purple varieties of passion fruits were slightly more affected by Fusarium wilt in the seedling stage compared to Yellow. As a preventive measure 2g/l Bavistin was applied to check the growth and attack of *Fusarium oxysporium.*

Unhealthy seedlings were removed from the nursery and seedlings were destroyed through incineration. Proper fumigation of Mist chamber was done to reduce the disease incidence.

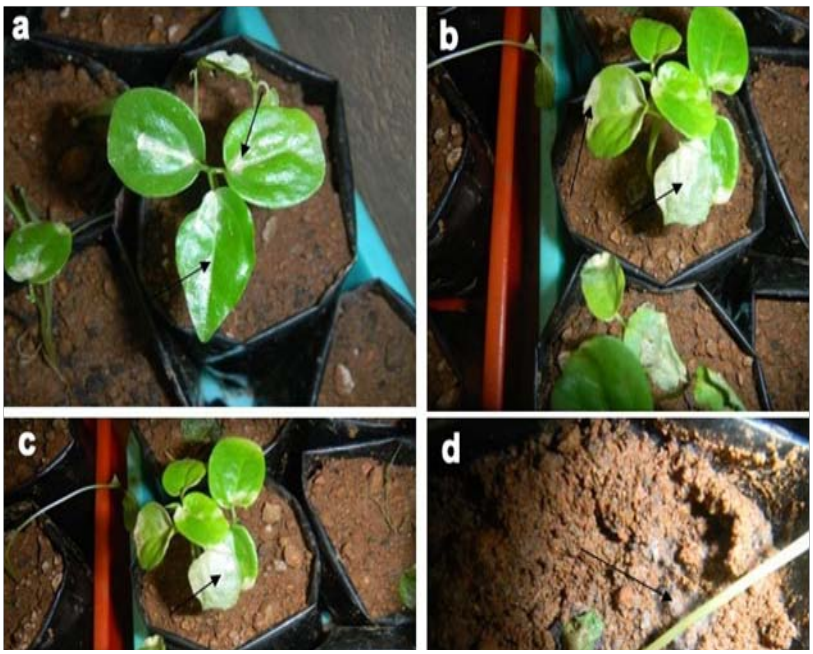


Figure 59 (a) Early symptoms of wilting on passion fruit leaf. (b) Yellow colored patches on passion fruit leaves. (c) Destruction of passion fruit leaves due to Fusarium wilt. (d) White cottony growth of Fusarium colony on passion fruit poly bag soil surface.

Land preparations for planting of passion fruit vines were done using JCB. Entire land was ploughed with extra caution for not removing the top fertile soil. Trellis was constructed by the mid week of April 2012. Immediately after trellis construction entire field was cleared to keep the rodents away. Proper weedicides application was done to keep the field ready for planting. Glycophan 40ml/l was used for chemical weeding. The experimental area was tilled and planting furrows (50 cm deep) was made at a distance of 1.5 m, 7 days before planting. Potting mixture was applied in the furrow to ensure nutrient supply to the young plants.

Figure 60 (A) Original field before land preparation (B) Field Clearance with JCB (C) Trellis Construction (D) Experimental Plot cleared and trellis constructed ready for planting

Figure 61 (A) Tilling of land for planting of passion fruit (B) Planting of passion fruit vine (C) Shading of passion fruit to avoid sunburn (D) Passion fruit plants with shading in the field (E, F) Passion fruit vines after one month of planting

Planting of passion fruit vines was done by the mid-week (13-07-2012) of July. 14 accessions from different regions of Kerala were planted in a randomized block design with three replications. Planted vines included nine yellow varieties (35Y, 45Y, 51Y, 55Y, 57Y, 66Y, 86Y, 88Y and125Y) and five purple varieties (134P, 142P, 143P, VkmP and KAVERI). Proper shading was given to avoid sunburn. Proper irrigation and fertilizer application (15g Urea +10g Potash/Plant) was done on a quarterly basis.

Figure 62. Passion fruit Vines two Months after Planting

Figure 63. Passion fruit vineyard three months after planting

Figure 64. Passion fruit vines four months after

Figure 65. Passion fruit Vines five Months after Planting

Figure 66. Arial View of Passion fruit vineyard six months after planting

Figure 67. Watering of Passion vines

Figure 68. Training of passion vines to the trellises

Diseases and Pest Control Measures

Disease symptoms like that of fusarium wilt, collar rot and black spot were noted on various varieties of passion vines. Disease incidence was higher for 88- Yellow followed by 35- Yellow, 86-Yellow, 45-Yellow, 142-Purple and 51-Yellow. The accessions Vazhakulam –Purple followed by Kaveri –Purple and 125- Yellow recorded lower disease severity. Collar rot disease caused by

fusarium species showed high prevalence in the vineyard. Affected plants showed leaf colour change to pale green, wilt, defoliation, necrotic girdling of the plant collar and complete die back of the plant. As control measures, drenching of SAAF (2.5g/l) and Bavistin (3 g/l) were done on need basis to check the severity of the disease. Intense care was taken while handling the passion vines to avoid spreading of the disease. Thrips attack was noted in younger as well as older leaves of passion vines. Hilban (2.5ml/l) application was done for proper pest controlling.

Figure 69. Yellowish leaf due to fusarium wilt

Figure 70. Leaf Minor attack on passion leaf

Figure 71. Passion fruit leaf with Black spot disease

Figure 72. Passion fruit leaf attacked by thrips

Figure 73. Passion fruit root damaged with collar rot disease

Figure 74. Fusarium wilting of passion vine

Figure 75. Die back of passion vine

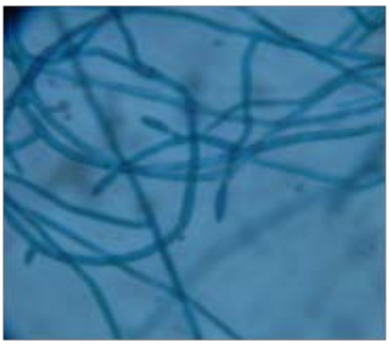


Figure76. Fusarium hyphae with spores identified from diseased sample

Figure 77. Fusarium spore identified from diseased sample

Table 41. Details of Plants died due to collar rot Disease

Sl. No	Accession	Replication	Plant No.	Symptoms	Disease	Pathogen	Remarks
1	88-Y	R3	2	Leaf colour changes to Pale green Leaf Wilt Defoliation Necrotic girdling of the plant collar Die back	Collar Rot	*Fusarium oxysporum*	Replanting of the lost plants have been done
2	88-Y	R3	3				
3	86-Y	R1	2				
4	86-Y	R3	1				
5	66-Y	R3	1				
6	45-Y	R3	4				
7	51-Y	R2	2				

a. New observations:

Growth parameters of the 14 superior selected accessions were recorded every three months. Results obtained till date highlight the following.

Table 42. Growth parameters of passion fruit vines three months after planting

No	Variety	Total length of Vines	Total No. of Branches	Total No. of Leaves	Total No. of Tendrils	Disease Incidence
1	125-Y	243.40	8.53	88.50	44.45	4.33
2	55-Y	226.40	5.94	111.75	35.83	4.67
3	88-Y	275.33	6.78	74.17	44.11	5.33
4	35-Y	199.77	6.03	57.50	30.15	3.67
5	86-Y	175.47	4.94	47.93	32.83	5.67
6	143-P	254.33	5.79	57.03	31.80	4.33
7	V-P	251.40	5.26	59.30	34.19	3.33
8	134-P	272.47	8.00	112.83	52.08	3.00
9	66-Y	249.90	9.07	123.67	57.86	3.67
10	45-Y	93.47	8.11	63.72	48.44	6.00
11	142-P	293.17	8.25	127.42	63.44	4.33
12	51-Y	248.35	7.83	113.97	71.03	4.00
13	57-Y	222.45	6.67	84.78	39.55	3.33
14	KAVERI	263.87	7.64	101.75	35.44	3.67
	G.Mean	234.56	7.06	87.45	44.40	4.24
	SEm	24.825	1.143	19.193	9.240	0.453
	CD	72.164	NS	NS	NS	1.317

At three months after planting, among the various growth parameters recorded, number of branches, number of leaves and number of tendrils did not show any variations with the varieties. However the length of vine showed significant variations with the varieties. The accession number 142-Purple followed by 88- Yellow had the highest vine length which was significantly higher than that of 45-Yellow, 86–Yellow and 35 Yellow, but statistically on par with all other varieties. The accession 45- Yellow recorded the least vine length. Though disease symptoms like that of fusarium wilt, collar rot and black spot were noted, the varieties did not show significant variations with regard to the disease severity as indicated by the disease score values.

Table 43. Growth parameters of passion fruit vines six months after planting

No	Variety	Total length of Vines	Total No. of Branches	Total No. of Leaves	Total No. of Tendrils	Disease Incidence
1	125-Y	638.67	59.25	16.41	13.11	4.33
2	55-Y	503.97	57.00	17.83	10.11	4.67
3	88-Y	558.08	48.17	17.36	9.95	6.33
4	35-Y	531.45	43.53	14.60	11.26	5.67
5	86-Y	440.20	35.42	16.81	13.17	5.67
6	143-P	702.92	47.42	16.01	12.48	5.33
7	V-P	513.90	41.67	20.01	16.69	3.33
8	134-P	661.75	48.33	12.79	11.88	4.67
9	66-Y	542.27	45.18	18.60	15.37	5.33
10	45-Y	436.78	39.20	14.08	12.35	5.67
11	142-P	692.23	44.08	16.42	13.40	5.67
12	51-Y	593.93	58.64	18.12	12.71	5.67
13	57-Y	531.00	40.08	16.19	12.45	5.33
14	KAVERI	613.52	53.20	15.53	11.60	3.67
	G.Mean	568.56	47.72	16.53	12.60	5.10
	SEm	56.898	5.556	1.655	1.367	0.566
	CD	165.397	NS	NS	NS	1.646
	CV%	17.439	20.576	17.259	18.667	19.245

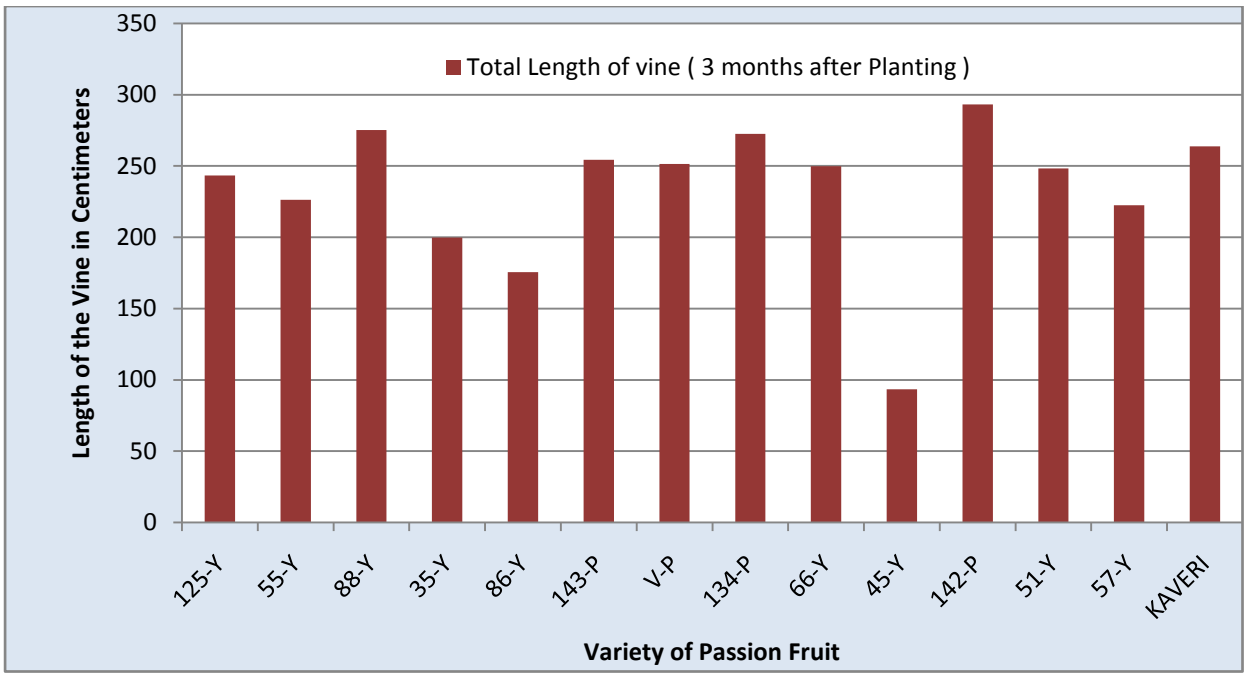


Figure 78. Comparison of total length of passion vines 3 and 6 months after planting

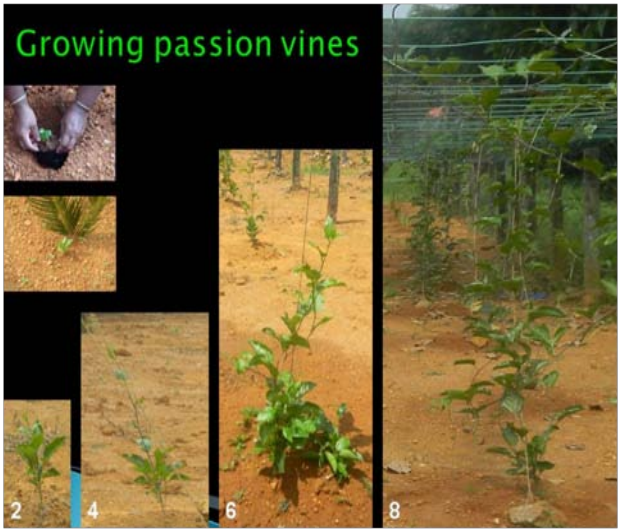


Figure 79. Growing vines 2, 4, 6 and 8 months after planting

Figure 80. Passion flowering and fruiting

At six months after planting, again the number of branches, number of leaves and number of tendrils did not vary with the varieties. However varieties showed significant variations in the length of the vines. The accession 143-Purple followed by 142- Purple and 134-Purple recorded higher length of vines. The accessions 45-Yellow and 86-Yellow recorded the least length of vines. Disease symptoms like that of fusarium wilt, collar rot and black spot were noted on the experimental plants and the disease severity showed significant variations as indicated by the score values. The accessions Vazhakulam –Purple followed by Kaveri –Purple and 125- Yellow recorded lower disease severity. The disease severity was highest for 88- Yellow followed by 35-Yellow, 86-Yellow, 45-Yellow, 142-Purple and 51-Yellow.

3. RESEARCH ON BANANA

3.1 Media standardization for the promotion of multiplication of banana

Objective

To formulate a new media for promoting multiplication and reduce rooting during subculture

Technical programme

The experiment was conducted at subculture stages. The callus cultures were inoculated to Murashige and Skoog medium (MS) supplemented with different concentrations and combinations of cytokines and auxins. The cultures were observed for a period of 21 days with an interval of seven days.

Result & Discussion

The sub cultured nendran inflorescences in the medium MS+ 0.5mg/L IAA + 5 mg/L BA(j) showed the maximum response with increased bud proliferation.

The sub cultured Poovan cultures showed best responses in the medium MS+ 2 mg/L NAA + 0.5 g/l Charcoal (q).

The sub cultured Red banana cultures showed the best response in the media MS+ 0.5mg/L IAA + 5 mg/L BA (j)

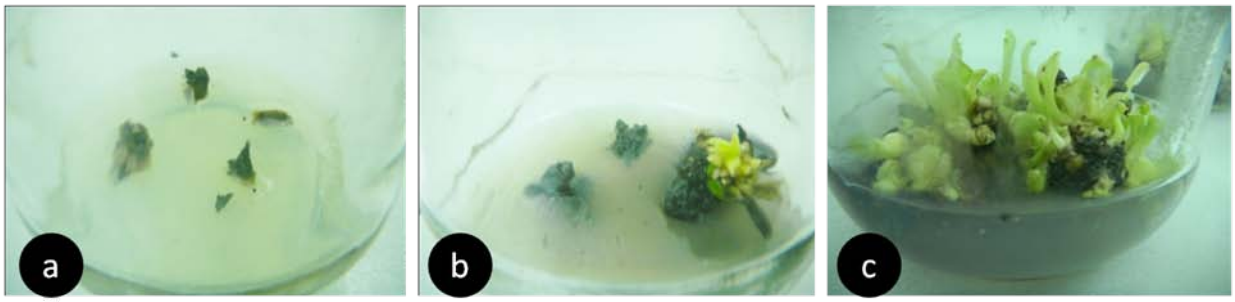


Figure 80. Effect of varying concentrations of cytokines&auxins on bud proliferation of nendran inflorescence. a - No change (Medium c) b - 1 bud (Medium d) c - 20 buds (Medium j)

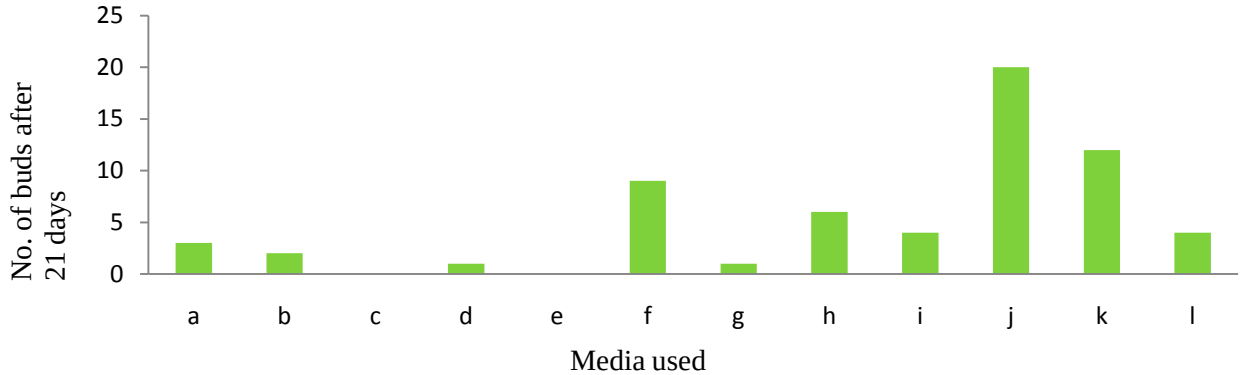


Figure 81. Effect of varying concentrations of cytokines & auxins on bud proliferation of nendran inflorescence

Table 44. Effect of varying concentrations of cytokines & auxins on bud proliferation of nendran inflorescence

Media used	After 7 days	After 14 days	After 21 days	% Of Recovery	Response
a (MS+ 1.5mg/L IBA + 4 mg/L BA)	No Change	3 Buds	3 buds	80	+
b (MS+1.5mg/L IBA + 3 mg/L BA)	1 bud	2 buds	2 buds	60	+
c (MS+1.5mg/L IBA + 2 mg/L BA)	No Change	No change	No change	80	-
d (MS+1 mg/L IBA + 4 mg/L BA)	No Change	1 bud	1 bud	60	+
e (MS+ 1 mg/L IBA + 3 mg/L BA)	No Change	No change	No change	60	-
f (MS+ 1mg/L IBA + 2 mg/L BA)	No Change	7 buds	9 buds	80	+ +
g (MS+ 0.5mg/L IBA + 5 mg/L BA)	No change	1 bud	1 bud	100	+
h (MS+ 0.5mg/L IBA + 4 mg/L BA)	No change	3 buds	6 buds	100	+ +
i (MS+ 0.5mg/L IBA + 3 mg/L BA)	No change	2 buds	4 buds	80	+
j (MS+ 0.5mg/L IAA + 5 mg/L BA)	8 buds	12 buds	20 buds	100	+ + +
k (MS+ 0.5mg/L IAA + 4 mg/L BA)	6 buds	8 buds	12 buds	80	+ +
l (MS+ 0.5mg/L IAA + 3 mg/L BA)	No change	3 buds	4 buds	80	+

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

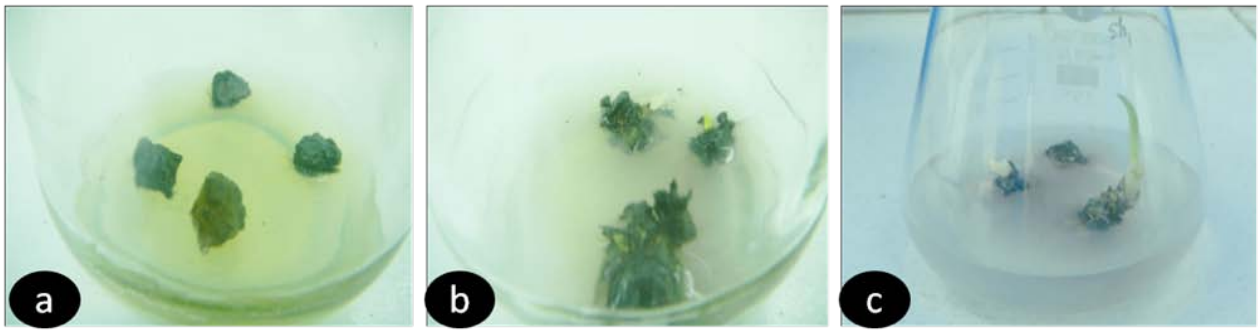


Figure 82. Influence of various combinations of hormones on multiplication of poovan. a - No change (Medium f) b - One bud (Medium j) c - One shoot & One bud (Medium q)

Table 45. Influence of various combinations of hormones on multiplication of poovan

Media used	*After 7 days*	*After 14 days*	*After 21 days*	*% Of Recovery*	*Response*
(j)MS+ 0.5mg/L IAA + 5 mg/L BA	No change	No change	1 bud	80	+
(f)MS+ 1mg/L IBA + 2 mg/L BA	No change	No change	No change	75	-
(q)MS+ 2 mg/L NAA + 0.5 g/l Charcoal	No change	No change	1 bud, 1 shoot	100	+ +

Negligible response (-); Minimum response (+); Medium response (+ +)

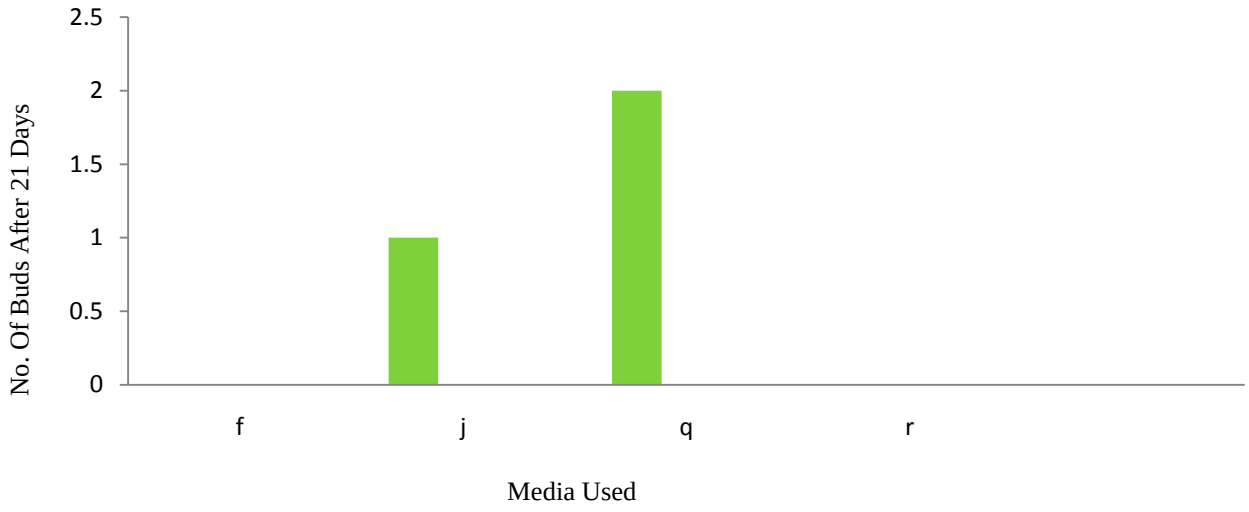


Figure 83. Influence Of Various Combinations Of Hormones On Multiplication Of Poovan

Table 46. Periodical response of various media combinations of red banana

Media used	*After 7 days*	*After 14 days*	*After 21 days*	*% Of Recovery*	*Response*
(j)MS+ 0.5mg/L IAA + 5 mg/L BA	Redding of callus	2 buds	3 buds	100	+ +
(f)MS+ 1mg/L IBA + 2 mg/L BA	1 bud	2 buds	1 shoot	100	+
(q)MS+ 2 mg/L NAA + 0.5 g/l Charcoal	No change	No change	No change	75	-
(r)MS+ 5 mg/L BA + 2 mg/L IAA	No change	No change	1 bud	100	+

Negligible response (-); Minimum response (+); Medium response (+ +)

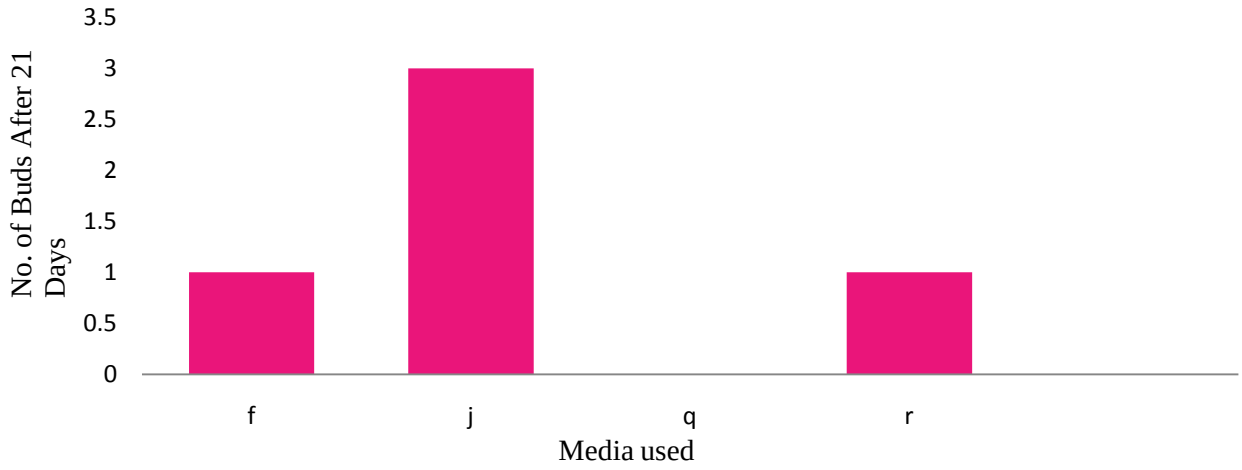


Figure 84. Periodical response of various media combinations of red banana

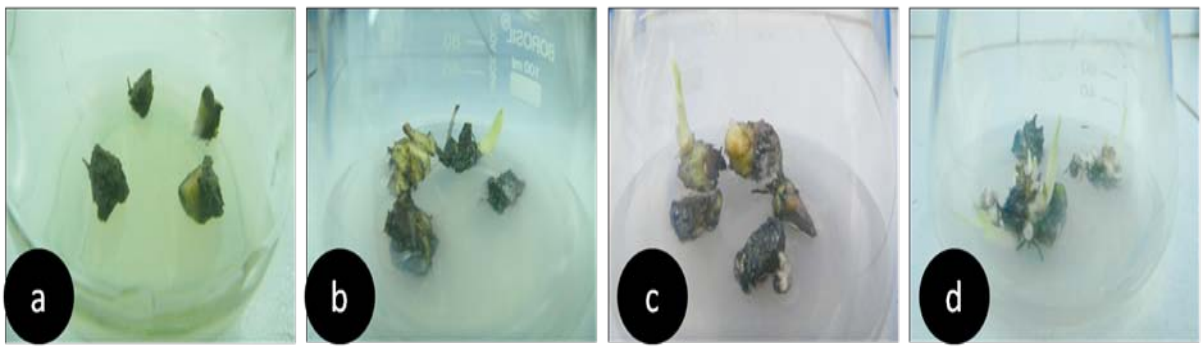


Figure 85. Periodical response of various media combinations of red banana. a - No change (Medium q) b - One bud (Medium r) c- One shoot (medium f) d- 3 buds (Medium j)

4. MASS PRODUCTION & SALE

The plant varieties we have in mass production category are tissue culture banana varieties such as Nendran, Red Banana, Poovan and Njali Poovan, pineapple varieties such as MD2, MTS, Amrutha and Kew. Tissue culture (MD2 and banana) plants are also cultured as 10 plants per bottle for sale. The passion fruit varieties are mainly mass produced via seeds. We are also strengthening the production of rooted cutting for passion fruit varieties such as giant, purple and yellow.

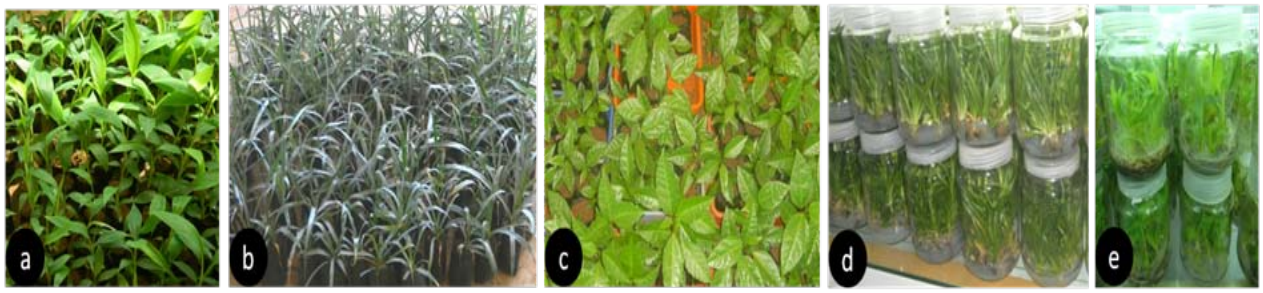


Figure 86. (a),(b) & (c) Tissue culture banana plants, pineapple and passion fruit plants in rooftop nursery (d) Ten plants per bottle – MD2 (e) Ten plants per bottle – Banana

The planting materials are sold in the form of TC bottles, poly bag plants and also as plants.

Figure 87. Plant Sale (a) sale of polybag plants (b) sale of bottle plants (c) sale of MD2 tissue culture plants separated from polybags

Planting Material Production

Table 47. Planting material production, receipt, target, etc for 2012-13

Crop/Variety	Target (No.)	Production (No.)	Unit Price (Rs.)	Sale (No.)	Receipt (Rs.)	Stock balance (No.)	Target for 2013-14 year (No.)
Pineapple TC	2,000	514	10	60 437	570 (5%) 4,370	1,203	2,000
Pineapple TC bottle with 10 plantlets		325	70	100	7,000	225	1,000
Passion fruit seedlings	2,000	2,762	5	740	3,700	2,022	2,000
Passion fruit seedlings (from Dec 10/12/2012onwards)			10	978	9,780		
Passion fruit TC & Rooted Cuttings	500	25	10	8	80	18	500
Banana TC	2,000	1,693	15	440 1,161	6,270 (5%) 1,7415	34	2,000
Banana TC Nendran (from Dec 10/12/2012 onwards)			20	57	1,140		
Banana TC bottle with 10 plantlets	1,200	883	100	562	48,919	121	1,200
Banana TC Nendran bottle with 10 plantlets (from Dec 10/12/2012onwards)			115	200	21,850(5%)		
Total	**7,700**	**6,202**	**355**	**4,698**	**1,21,094**	**3,885**	**7,700**

Station Receipt and Expenditure

Table 48. Station Receipt and Expenditure

Project	Budget Estimate (Rs.lakh)	Revised Estimate (Rs.lakh)	Expenditure (Rs.)	Receipts (Rs.)
0034: Non-Plan	15.82	23.90	2168012	
3370: Research on pineapple	10.56	04.65	454550	
3500: Research in passion fruit	02.30	01.30	124725	
9027: KSCSTE SRS - Evaluation of Passion fruit types for commercial cultivation in Kerala	05.65	05.65	534904	
Total	34.33	35.50	3282191	142022 *

* Out of the Rs.1,42,022 receipt, Rs. 55,622 is pending from KVK, Kumarakom. Rs.1,00,000 was transferred to comptroller by cheque No. 805618 dt. 23.01.2013.

5. EXTENSION ACTIVITIES

The beneficiaries of various SHM projects under different Krishibhavans in Ernakulam (dist) were selected in a meeting held on 10th Dec. 2012 at the Chamber of Deputy Director of Horticulture, Civil Station, Kakanad

5.1 Trainings Attended

Figure 88. Training Programmes (a) Ms. Renju, Research Assistant, PRS, Vazhakulam discussing banana tissue culture production at BRS , Kannara (b) Ms. Anjana R., Research Assistant, PRS, Vazhakulam, taking class on Plant Tissue Culture & overall activities of the station to VHSE students (c) Dr. P.P. Joy attending farmers queries from Tamil nadu (d) Field demonstration of Honda Brush Cutter at NAPF

Table 49. Training Attended

Date	Trainings Attended
28/06/2012	Ms. Anjana R., Ms. Renju Rose Kurian deputed for training on virus indexing at Banana Research Station , Kannara.,Thrissur for two days and submitted a detailed report to the station.
27/11/2012	Ms. Anjana R., Ms. Soumya K. K., one day training in Phytochemistry lab techniques at AMPRS, Odakkali

5.2 Training Programmes Organized

Table 50. Training programmes organized

Date	Training Programmes Organized
13/04/2012	Training given to 30 farmers on passion fruit and pineapple cultivation. They were accompanied by Sri. K. Elakkunanan Agriculture Officer & Sri. C. Verma, Assistant Agriculture Officer under ATMA scheme, Tamil Nadu
21/05/2012	Training on pineapple cultivation offered to pineapple farmers & department officials in the Pineapple Mission meating preceded by Sri. Joseph Vazhakkan M.L.A in which Dr. Pratapan, Director SHM & Mr. Pushpangadan V. V., CEO, VFPCK participated
09/07/2012	Training a field demonstrative of Honda Brush Cutter was organized at NAPF for acquainting with the use and maintenance of brush cutters. The demonstration was made by Honda Brush Cutter agency, Muvattupuzha.
12/09/2012	Training on pineapple cultivation was imparted to 50 horticultural crop growers accompanied by Sri. S. Ayyaswamy, Assistant Director Horticulture, Tamil Nadu.
27/09/2012	A seminar on judicious application of fertilizers in pineapple was organized jointly by Indian Potash Limited, Rubber Point & KAU. A class on judicious fertilizer application in pineapple was taken by Dr. P. P. Joy, Asso. Prof. & Head, PRS, Vazhakulam during the seminar. The seminar was attended by more than 100 pineapple farmers.
20/10/2012	Training on technical aspect of Tissue culture, Phytochemistry- fruit analysis and Pathology was given to 50 VHSE students from Govt. VHSE , Thodupuzha. They were introduced to the advanced equipments in the labs. Training sessions were conducted by Ms. Anjana R., Ms. Renju Rose Kurian & Ms. Marians Paul, Research Assistants, PRS, Vazhakulam
01/11/2012	A seminar was organized by the Vazhakulam Agriculture Club on the *Kerala Piravi Dinam.* A class on pineapple cultivation was also taken during the seminar.
02/11/2012	A seminar was organized by Krishibhavan, velliyamattom under ATMA Scheme. A class on paddy & pineapple cultivation was handled by Dr. P. P. Joy, Asso. Prof. & Head, PRS, Vazhakulam, during the seminar.

5.3 Media Coverage

Figure 89. Media coverage (a) *metro vaartha*, 05.01.2012, p. 14 (b) *Malayala Manorama* CHN, 24.06.2012, p.16 (c) Kerala Karshakan - Fruit Basket,March 2013, p.46-47 (d) *Malayala Manorama* CHN, 28.06.2012, p.16 (e) *Mangalam* CHN, 18.11.12 (f) *Krishiyanganam*- Cover story March 2013 p.34-36 (g) *Krishiyanganam* - Cover story March 2013 p.18-20

5.4 Publications

The following are the publications brought out during the year.

Joy P. P. and Sindhu G. 2012. DISEASES OF PINEAPPLE (Ananas comosus): Pathogen, symptoms, infection, spread & management. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala, India.

Joy P. P. and Sherin C.G. 2012. DISEASES OF PASSION FRUIT (Passiflora edulis): Pathogen, Symptoms, Infection, Spread & Management. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala, India.

Joy P. P. and Sherin C.G. 2012. INSECT PESTS OF PASSION FRUIT (Passiflora edulis): Hosts, Damage, Natural Enemies and Control. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala, India.

Joy P. P. and Minu Abraham. 2013. FRUITS, BENEFITS, PROCESSING, PRESERVATION AND PINEAPPLE RECIPES. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala, India.

Joy P. P., Anjana R. and Prince Jose. 2013. PROTOCOL FOR MICROPROPAGATION OF PINEAPPLE (MD-2). Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686670, Muvattupuzha, Ernakulam, Kerala, India

Joy P. P., Anjana R. and Prince Jose. 2013. PROTOCOL FOR MICROPROPAGATION OF BANANA. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686670, Muvattupuzha, Ernakulam, Kerala, India

Joy P. P. and Soumya K.K. 2013. BASIC FRUIT ANALYSIS OF PINEAPPLE: A LABORATORY MANUAL. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686670, Muvattupuzha, Ernakulam, Kerala, India

Soumya K.K. and Joy P. P. 2013. Recent Trends in Biology. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686670, Muvattupuzha, Ernakulam, Kerala, India

Joy P. P. 2013. Pineapple- Cultivation and utilisation. *Krushiyankanam* 17(4, 5): 18-22

Joy P. P. 2013. Passion fruit- Cultivation and utilisation. *Krushiyankanam* 17(4, 5): 34-38

Joy P. P. 2013. Passion fruit. *Kerala Karshakan* 58(8):46-47

Full text of these publications are given in appendix and also available at the station websites.

5.5 New Project Proposal

A meeting of Pineapple Mission was conducted at the chamber of honourable minister for Agriculture Sri. K.P. Mohanan, at Assembly Complex, Thiruvananthapuram on 25/07/2012 and discussed the future line of action of the Mission. The body suggested to submit a research & development proposal for the comprehensive development of the sector and increasing the production and productivity of pineapple in the state. Accordingly a project proposal under Pineapple Mission entitled " *Development of Pineapple Sector in Kerala in Mission Mode*" at a budget of Rupees 137.8 lakh for three years was submitted to the Govt. Of Kerala through the Director Of Extension, KAU on 28/07/2012 with objective of boosting the production and productivity of superior quality GI registered Vazhakulam Pineapple in Kerala through comprehensive multipronged integrated approach in mission mode. The full text of the project proposal is given in the appendix.

6. VISITORS

Table 51. Visitors to the station during the year 2012-13

Date	Visitors
13/04/2012	K. Elakumaran Agri. Officer, Padmanabhapuram, Tamil nadu
27/04/2012	Mr. S. Rajkumar, Territory Manager, Mr. A. Joseph, Office Trainee, Mr. Joseph Chacko, Technical assistant, Biostadt India Limited, Mumbai.
02/05/2012	IFFCO, Kerala
03/05/2012	Dr. Raji P, Associate Professor of Plant Pathology, RARS, Pattambi.
22/05/2012	Dr. Jim Thomas, Professor and Head, Directorate of Extension, Mannuthy.
22/05/2012	Dr. Anitha Cheriyan. K, Professor(Plant Pathology) BRS, Kannara.
23/05/2012	Dr. I. Johnkutty ADR, Pattambi & Dr. Gracy Mathew, Assoc. Prof. Agronomy, AMPRS, Odakkali conducted interview
25/06/2012	Mr. Sandeep Jose, Asst. Manager, Agri Business Centre, Axis Bank, Cochin discussed on the scope of fibre extraction from pineapple
02/07/2012	Mr. George Kurian Manager, Mountain fruits, Mundakayam, Idukki.
04/07/2012	Mr. P. Gopalkrishnan, Stabaka.com & Sriman, HML
24/07/2012	Mr. Vinoy K Menon, Area sales Manager, Rallis India ltd.
26/07/2012	Mr. Anson C. J. , Research Fellow, inter university centre for IPR studies, Cusat, Cochin.
27/07/2012	Mr. K. Induchoodan, Area sales officer, Indian Potash Ltd. Mr. Biju C George, Sales Manager, Rubber Point, Thodupuzha.
01/08/2012	Mr. Paul Mathew ,Thottappillil, Puthencruz
10/08/2012	Mr. Shilu. C. John, Area Field manager, Indofil Indusries Ltd.
17/08/2012	Mr. James George, Secratary, Pineapple farmers Association (P. F. A)
17/08/2012	Mr. Jose Kalapura, President, P. F. A.
07/09/2012	Mr. Induchoodan K, Area sales officer, Indian Potash Ltd,Mr. Biju C George, Sales Manager, Rubber Point, Thodupuzha.Mr. Tony, area manager, IPL.
12/09/2012	50, Horticulturacrop growing farmers with Sri, S. Ayyapasamy , Tamil nadu
24/09/2012	Mr. Lijin Joy, Thanathuparambil,Kavana, farmer
20/10/2012	Teachers & Students , Govt. Vocational higher Secondary School, Thodupuzha.
27/10/2012	Mr. Pushpangadan V. V., CEO, VFPCK
27/10/2012	Patric Godino, Coral, Fort Cochin
31/10/2012	Mr. Prasanth, Research Fellow, North Campus, Delhi University
03/11/2012	Mr. Tomy Joseph, Koothattukulam Mr. K. G. Kuriankutty, Piravom
03/11/2012	Mr. K. K. Mohanan, Member, Koothattukulam Gramapanchayath.
06/11/2012	Dr. Prashant Butt, Sun Agrigenetics Pvt.Ltd, Vadodhara, Gujarat
22/12/2012	Mr. Mahesh V, Cropx Biochemicals, Cochin
13/02/2013	Dr. Lyla K. R. , Professor & Head, AICRP on BCCP&W, COH, Vellanikara, Mrs. Vidhya C. V. , Asst. Prof. AICRP on BCCP&W,COH, VKA, Mr. George A. X. , Farm manger, AICRP on BCCP&W, Mr. Sunish M. S. , Farm Officer, AICRP on BCCP&W, Ms. Aswathy Vijayan, MSc (Ag) Student. COH.
18/02/2013	Mr. Narendra Mohan, Nodel Officer, PHM, Marketing, Govt. Of Bihar.
18/03/13	Mr. Arun Mandal & Team from W. Bengal, Pineapple Growers Association
23/03/13	Mr.Haris & Abdul Lathif, Kollam, haris8796@hotmail.com

Dr. Lyla K. R. , Professor & Head & Mrs. Vidhya C. V. , Asst. Prof. AICRP on BCCP&W,COH, VKA & team visiting the station to tackle mealy bug in pineapple

Arun Mandal & team, Pineapple Growers Association, West Bengal visiting the station and the rooftop nursery

Dr. Jim Thomas, Professor and Head, DOE, Mannuthy & Dr. Anitha Cheriyan. K, Professor (Plant Pathology) BRS, Kannara discussing pests and disease issues in pineapple

Mr. P. Gopalkrishnan, Stabaka company along with Sriman, HML, discussing with Head of the station

VHSE students from Govt. Vocational Higher Secondary School, Thodupuzha attending training sessions at the station

Dr. Shyla Raj, Prof. Biotechnology, RRS, Vytilla, discussing tissue culture activities with the station Head

Figure 90. Visitors to the station during the year

Mr. James George, Secratary & Mr. Jose Kalapura, President Pineapple farmers Association discussing with Head of Station

Mr. P. Gopalkrishnan, Stabaka & Sriman, HML, discussing Agri business

Dr. I. Johnkutty ADR, Pattambi along with Head of the Station interviewing the candidate for KSCSTE Project

Head of the station interacting with passion fruit growers

Mr. Narendra Mohan, Nodal Officer, PHM, Marketing, Govt. of Bihar. discussing with Head of the Station

Mr.Haris & Abdul Lathif discussing pineapple export to Iran

Figure 90. Visitors to the station during the year (Continued)

Mr. Pushpangadan V. V., CEO, VFPCK with PRS team while visiting the station

Farmers from ATMA, Tamil nadu visiting the station

Entomology P.G student from KAU inspecting the pineapple field & collecting mealy bug infested samples from the field

Dr. Prashant Butt, Sun Agrigenetics Pvt.Ltd, Vadodhara, Gujarat discussing on commercial tissue culture production

Mr. George Kurian Manager, Mountain fruits, Mundakayam, Idukki, interacting with Head of the station

PRS Team 2012-13

Figure 90. Visitors to the station during the year (Continued)

7.1 Diseases of pineapple (*Ananas comosus*): pathogen, symptoms, infection, spread & management

DISEASES OF PINEAPPLE (*Ananas comosus*)

Pathogen, symptoms, infection, spread & management

Joy P. P. & Sindhu G., Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala, India, Tel. & Fax: +914852260832, Email: prsvkm@gmail.com

Pineapple is one of the most important fruit crops of Kerala. The pineapple originated in South America, where native people selected a seedless mutation from a wild species. It belongs to the family Bromeliaceae, many members of which are epiphytes living on trees and rocks. Pineapples grow in the soil and resemble epiphytes in that their roots are intolerant of poor soil aeration. ‘Kew’ of the smooth-leaf 'Smooth Cayenne' group and ‘Mauritius’ of the rough leaf 'Queen' group are the two varieties of pineapple grown in India. Diseases of pineapple are associated with fungi, bacteria, nematodes and viruses. Pineapple roots are adventitious and will not regenerate if damaged. Mealy bug wilt also affects the root system. Base rot and water blister are economically significant. Diseases such as Phytophthora fruit rot, pink disease, yeasty rot and marbling at times become significant warranting control measures though they occur infrequently and have only a minor effect on yield or fruit quality in general.

■ FUNGI ASSOCIATED DISEASES

■ PHYTOPHTHORA HEART (TOP) ROT

Pathogen

The oomycetes *Phytophthora cinnamomi* and *Phytophthora nicotianae*

Symptoms

- Plants of all ages are attacked, but three to four month old crown plantings are most susceptible.
- Fruiting plants or suckers on ratoon plants may be affected.
- The colour of the heart leaves changes to yellow or light coppery brown. Later, the heart leaves wilt (causing the leaf edges to roll under), turn brown and eventually die.

Plant collapse caused by Phytophthora heart rot

- Once symptoms become visible, young leaves are easily pulled from the plant, and the basal white leaf tissue at the base of the leaves becomes water-soaked and rotten with a foul smell due to the invasion of secondary organisms. The growing point of the stem becomes yellowish-brown with a dark line between healthy and diseased areas.

Infection and spread

Chlamydospores of the two species are the primary inoculum and they can survive in the soil or in infected plant debris for several years. They germinate directly to produce hyphae that are able to infect roots and young leaf and stem tissue, or indirectly to produce sporangia.

Phytophthora pathogens are soil inhabitants and require water for spore production and infection. As free water is required for producing sporangia and releasing motile zoospores, infection and disease development is exacerbated in soils with restricted drainage.

Rotten pineapple heart, leaves and fruit caused by Phytophthora heart rot

Management

Use systemic fungicides to reduce heart rot. This program should start with the treatment of planting material before planting. After planting, drenching or spraying with registered fungicides at recommended rates and intervals is necessary to ensure against losses. Infected plants can be saved only if treated soon after symptoms appear.

Avoid excessively deep planting and prevent soil entering the hearts during planting.Well-drained soils are essential for minimizing the risk of Phytophthora infection. This can be achieved through careful field selection, planting on raised beds at least 20 cm high, constructing drains to intercept run-off before it reaches the plantation, constructing drains within the field so that water is removed rapidly without causing erosion and installing underground drains.

Phytophthora cinnamomi becomes more active as soil pH levels increase above 4.0. Liming materials, which raise pH, should be used cautiously. *Phytophthora nicotianae* tends to be more active in soils with higher nutrient status. Sulfur may be used to reduce pH in soils with a pH above 5.5, but this is not a replacement for other management practices.

■ PHYTOPHTHORA ROOT ROT

Pathogen

The oomycete of *Phytophthora cinnamomi*

Symptoms

Roots destroyed by *Phytophthora cinnamomi* (right) compared with a healthy root system (left)

- The symptoms above ground are similar to those caused by nematodes, mealy bug wilt and low levels of soil oxygen and are not diagnostic. Leaves change in colour from a healthy green through various shades of red and yellow.
- Leaf tips and margins eventually become necrotic, the root system is dead and plants can easily be pulled from the ground.

- Fruits from infected plants colour prematurely become small and unmarketable. If symptoms are recognized early and control measures are taken plants can recover. If roots are killed right back to the stem, they often fail to regenerate.

Infection and spread

Losses can be severe in poorly drained fields. Plants on even relatively well-drained soils can be affected during prolonged wet weather. Losses from root rot can be serious in high rainfall areas where prolonged rains extend into the winter months. The disease can eliminate the ratoon crop. Rough leaf varieties and some low acid hybrids are more susceptible than Smooth Cayenne.

Management

Use systemic fungicides to reduce heart rot. This program should start with the treatment of planting material before planting. After planting, drenching or spraying with registered fungicides at recommended rates and intervals is necessary to ensure against losses. Infected plants can be saved only if treated soon after symptoms appear.

Avoid excessively deep planting and prevent soil entering the hearts during plạnting. Well-drained soils are essential for minimizing the risk of Phytophthora infection. This can be achieved through careful field selection, planting on raised beds at least 20 cm high, constructing drains to intercept run-off before it reaches the plantation, constructing drains within the field so that water is removed rapidly without causing erosion and installing underground drains.

■ BASE (BUTT) ROT

Pathogen

The fungus *Chalara paradoxa*

Symptoms

- Symptoms are seen only on crowns, slips and suckers before or immediately after planting. A grey to black rot of the soft butt tissue develops, leaving stringy fibers and a cavity at the base of the stem. If affected material is planted, partial decay of the butt severely reduces plant growth
- When butt decay is severe, plants fail to establish, wilt rapidly and leaf tissue dies. Unlike Phytophthora heart rot, the young leaves remain firmly attached to the top of the stem. Infected plants can easily be broken off at ground level.

Infection and spread

The fungus is important in the breakdown of pineapple residues after cropping and survives as chlamydospores in soil and decaying pineapple residues. The fungus commonly infects plants through fresh wounds occurring where the planting material has been detached from the parent plant and destroys the soft tissue at the base of the stem. Material removed during showery weather and stored in heaps is particularly prone to infection. Tops (crowns) used for planting are particularly susceptible. Conidia are produced under conditions of high humidity and can be dispersed by wind. Losses of planting material and plantings from diseased material can be severe at times.


Base (butt) rot disease destroys the soft tissue at the base of the pineapple stem

Management

Do not leave a portion of fruit attached to the crown when picking. Treat material to be planted with a recommended fungicide immediately after removal (without drying). Store planting material on top of plant rows in a single layer with the butts exposed to the sun, or laid them on the ground in a similar manner. Losses are reduced greatly by curing the planting material base. If prolonged wet weather occurs, spray upturned butts or dip with a recommended fungicide within five hours of harvesting. Improve soil drainage and avoid planting during wet weather.

■ FRUITLET CORE ROT (GREEN EYE)

Pathogen

The fungi *Fusarium guttiforme* and *Penicillium funiculosum*

Symptoms

- This is an internal fruit disease. Smooth Cayenne fruits do not usually show any external symptoms. However, fruit of the rough-leaf (Mauritius) may produce fruitlets that fail to colour – a condition often referred to as 'green eye'.
- Severely affected fruitlets may become brown and sunken as the fruit ripens. Internal symptoms consist of a browning of the centre of the fruitlets starting below the floral cavity and sometimes extending to the core. The browning, which remains quite firm, varies in size from a speck to complete discolouration of one or more fruitlets.

Infection and spread

Penicillium funiculosum infects the developing fruit at some stage between initiation and open flower. Infection is favoured by cool temperatures (16–20^{o}C) during the five weeks after flower initiation, during which time the fungus builds up in leaf hairs damaged by mites. Similar cool temperatures are required for infection from about 10–15 weeks after flower induction. Symptoms of fruit let core rot on a fruit cylinder in damaged leaf hairs. *Fusarium guttiforme* enters the fruit through open flowers or injury sites. The risk of disease caused by this fungus is higher when flowers are initiated and fruit mature under warm conditions.

Pineapple fruitlet core rot (green eye) disease symptoms externally and internally

Management

Fungicides have not been effective except when applied directly into the opening of the terminal leaves that is created by the emerging inflorescence.

■ FUSARIOSIS

Pathogen

The fungus *Fusarium guttiforme*

Symptoms

- It is sporadic and affects all parts of the pineapple plant but is most conspicuous and damaging on fruit.
- Fruits exhibit stem rosetting and curvature of the plant because portions of the stem are girdled or killed.
- Rough leaf pineapple cultivars are more susceptible than smooth-leaf varieties.

Infection and spread

Infections of the inflorescence and fruit occur primarily via injuries caused by insects, particularly the pineapple fruit caterpillar (*Thecla basilides*) and by infected planting materials.

Fusariosis showing brown discolouration and gum exudates. Inset: symptoms in cut fruit, close-up of gum exudate at the base of the fruit

Management

The sporadic nature of the disease makes chemical control impractical and uneconomic. Fungicide and insecticide applications at flower induction and three weeks after forcing can reduce disease.

■ GREEN FRUIT ROT

Pathogen

The oomycete of *Phytophthora cinnamomi*

Green fruit rot

Symptoms

- Green fruit in contact with the soil are liable to be infected.
- A water-soaked rot develops internally behind affected fruit lets with no external symptoms, As the disease progresses, a general, water-soaked rot of green fruit with a distinct brown margin develops in green fruit.

Infection and spread

The pathogen lives in the soil and requires free water for spore production and fruit infection. Ratoon crop fruit lying close to or touching soil are most affected. Spores may be splashed by rain on to fruit near the ground.

Management

Apply systemic fungicides that are used to control root and heart rot, protecting the inflorescence and young fruit with fungicides. Although most strains of *F. guttiforme* cause fruitlet core rot, some strains cause fusariosis. Besides symptom development, there is no test available to distinguish the strains, so identification requires pathogenicity testing.

■ INTERFRUITLET CORKING

Pathogen

The fungus *Penicillium funiculosum*

Symptoms

- Fruits affected by inter fruitlet corking often show shiny patches on the shell early in their development, where the trichomes (hairs) have been removed by mite feeding.
- Externally, corky tissue develops on the skin between the fruitlets, but usually only 'patches' of eyes are affected.
- Fine, transverse cracks may also develop on the sepals and bracts.
- In moderate to severe cases, corkiness surrounding fruitlets prevents their development and one side of the fruit will be malformed.

Management

Inter fruit let corking is limited almost exclusively to fruit initiated in early autumn. It is sporadic and often confused with boron deficiency. Fungicides have not been effective except when applied directly into the opening of the terminal leaves that is created by the emerging inflorescence.

■ LEATHERY POCKET

Pathogen

The fungus *Penicillium funiculosum*

Symptoms

- Fruits do not usually show any external symptoms. Internally, the formation of corky tissue on the walls of the fruitlets makes them leathery and brown.

Infection and spread

See fruitlet core rot. Leathery pocket occurs sporadically. *Penicillium funiculosum* infects the developing fruit at some stage between initiation and open flower. Infection is favoured by cool temperatures (16–20^{o}C) during the five weeks after flower initiation, during which time the fungus builds up in leaf hairs damaged by mites. Similar cool temperatures are required for infection from about 10–15 weeks after flower induction.

Management

The sporadic nature of the disease makes chemical control impractical and uneconomic. Miticide applications at flower induction and three weeks after forcing can reduce disease.

■ WATER BLISTER

Pathogen

The fungus *Chalara paradoxa* , which also causes butt (base) rot and white leaf spot.

Symptoms

- Symptoms include water blister, which is also referred to as black rot or soft rot. This causes a soft, watery rot of the fruit flesh and makes the overlying skin glassy, water-soaked and brittle.
- The skin, flesh and core disintegrate and the fruit leaks through the shell. In advanced cases, this leaves a fruit shell containing only a few black fibres. This shell collapses under the slightest pressure.

Internal symptoms of water blister

Infection and spread

Infection occurs through shell bruises and growth cracks but mainly through the broken fruit stalks. The disease is most active in warm, wet weather and is most severe from January to April, when the summer crop is harvested. (The correlation between rainfall before harvest and disease after harvest has resulted in the name ‘water blister’). When fresh fruits are marketed with the crowns left on, this eliminates a major point of entry for the fungus..

This is the major postharvest disease of fruit for the fresh fruit market. The disease takes three to four days to develop after harvest and is therefore not a common problem in fruit used for canning. Water blister can be severe in fresh fruit consigned to distant markets when refrigeration is not available. The disease does not occur in the field unless fruits are over-ripe or injured.

Management

Handle fruit carefully to avoid bruising and scuffing. Rapid fungal invasion occurs through even minute, weeping fractures. Reject sun burnt and damaged fruit, because these often have minor skin cracks that are readily infected. Dip the base of the fruit in a recommended fungicide within five hours of harvesting and store fruit at 9^{o}C. This is most important for fruit harvested during warm, wet weather. Remove pineapple refuse and rejected fruit from in and around the packing shed. Treat the shed with the recommended disinfectant once a week.

■ WHITE LEAF SPOT

Pathogen

The fungus *Chalara paradoxa* , which also causes water blister and butt (base) rot.

Symptoms

- The first symptom is a small, brown spot on the leaf, usually where the leaf margin has been rubbed by another leaf during strong winds.
- These spots lengthen rapidly during wet weather. During prolonged wet periods, spots may reach more than 20 cm in length and spread to the leaf tip. Fine weather rapidly dries the affected area leaving cream coloured or almost white, papery spots; hence the name ‘white leaf spot’. The margins of the spot often remain brown.

White leaf spot symptoms appearing on a pineapple crop and a diseased leaf

Infection and spread

Chalara paradoxa is common in pineapple plantations. The fungus will only invade wounds and is most active in warm, wet weather.

Management

White leaf spot occurs commonly between March and May. The disease is of no economic significance. Management measures are rarely warranted.

■ FRUIT ROT BY YEAST AND CANDIDA SPECIES

Pathogen

The Yeast *Saccharomyces* spp. and *Candida* spp.

Symptoms

- Yeasts ferment sugar solution, producing alcohol and releasing carbon dioxide. The first symptom is a bubbling exudation of gas and juice through the crack or injury where infection occurred.
- The shell then turns brown and leathery and, as the juice escapes, the fruit becomes spongy.
- Internally, the decaying flesh turns bright yellow and develops large gas cavities. Finally, all that remains of the fruit is the shell and spongy tissue.

Yeasty rot externally shows gas bubbles and juice exuding through the skin and internally fermenting

Infection and spread

In spring, rapid changes in fruit growth, resulting from the shift from cold and dry to warm and wet weather, can result in the pineapple skin cracking between fruit lets. Fruit affected by even minor frost damage are prone to cracking as they ripen in spring. Yeasts immediately invade the juice weeping from those wounds, and these fruits are severely damaged or destroyed as they ripen. The disease may occur before or after harvest.

Management

Yeasts are among the most common organisms found in nature. Yeasty rot is widespread but occurs mainly during spring in overripe or damaged fruit. Protect fruit that will ripen in spring in frost-prone areas by covering young developing fruit with paper bags. Fruit showing even minor interfruitlet cracking should not be consigned to the fresh-fruit market. Any fruit showing fractures between fruitlets should be picked at the earliest stages of fruit maturity to minimize losses.

■ NEMATODES ASSOCIATED DISEASES

Pathogen

Root-knot nematode (*Meloidogyne javanica*), the root lesion nematode (*Pratylenchus brachyurus*) and the reniform nematode (*Rotylenchulus reniformis*)

Symptoms

- Root-knot nematodes produce distinct terminal swellings on the roots, stopping further root development. The root lesion nematode invades the outer root tissues, causing black areas (lesions) of dead or injured plant cells on the root surface.
- These lesions can completely encircle the root. Reniform nematodes reduce the number of lateral and fine feeder roots; the remainder elongate normally so that plants retain good soil anchorage. Root-knot nematodes cause stunting, yellowing and dieback of plants.

Life cycle

Juvenile root-knot nematodes invade roots near the tips. As these mature into females, the cells enlarge and develop into galls. When each female matures, it will lay some 2000 eggs in a small mass on the root surface. Between 25 and 30 days after the initial egg laying, juveniles invade the root. Root-knot nematodes produce many generations each year and soil populations can increase rapidly in optimal growing conditions. Root-lesion nematodes primarily live in the plant roots. They only enter the soil when migrating from one plant to the other. They move through the root cells, feeding on the cells and generally disrupting the physiological processes of the root. This nematode first moults inside the egg then passes through three juvenile stages before reaching adulthood. Both juvenile and adult nematodes can penetrate roots, so that infested roots contain all development stages: eggs, juveniles and adults. Reproduction occurs quickly in summer and each generation is completed in 29 to 45 days. Reniform nematodes are well adapted to warm dry conditions, and very high populations can develop very quickly. They have a wide host range, including cow peas and watermelons, which may be grown in rotation with pineapple. Unlike root-knot nematode, the reniform nematode does not have to feed when it hatches and can survive in fallow soil for long periods.

Management

Root-knot nematodes are the most damaging of all nematodes in field. Fruit yields can be markedly reduced, particularly in ratoon crops. Root lesion is common in all pineapple-growing

districts and high populations can reduce ratoon crop yields, but effects are often masked by symptoms caused by root-knot nematodes.

Most nematode populations, except reniform nematodes, decline rapidly in a weed-free or host-free fallow period. However, more than six months' fallow is needed for good results. For short fallows, keep the fields free from weeds. For longer fallows, plant inter-fallow crops that are not hosts for nematodes. Thorough land preparation will directly reduce nematode numbers; it will allow the soil to dry out and accelerate the breakdown of plant material that harbours nematodes. Use preplant soil sampling to assess the level of nematodes. If significant numbers are found, apply a registered nematicide before planting. In the plant crop, use nematode testing to determine nematode levels at six to eight months, and at 12 months, after planting. If significant numbers are found, apply a registered nematicide. Use nematode testing to assess the incidence of nematodes immediately after plant crop harvest and apply a registered nematicide if testing indicates the need for.

■ BACTERIA AND PHYTOPLASMAS ASSOCIATED DISEASES

■ MARBLING

Pathogen

The bacteria *Pantoea ananatis* and *Acetobacter* spp.

Symptoms

- Infected fruits do not show any external symptoms. Internally, the flesh is red-brown and granular and has a woody consistency.

Pineapple marbling disease showing red-brown granular flesh with woody consistency

Infection and spread

The disease occurs when flowers are initiated and when fruit mature under warm, wet conditions. The bacteria enter through the open flower and natural growth cracks on the fruit surface. Infected fruit are usually low in both acid and sugars.

Marbling is a minor problem that occurs sporadically. The disease is serious only in countries where pineapple fruit mature under lowland, tropical conditions.

Management

A practical way of managing marbling is not known. Internal symptoms are clearly visible in infected fruit, and fruit can be rejected easily during processing. Smooth Cayenne is moderately resistant.

■ PINK DISEASE

Pathogens

Bacteria *Pantoea citrea* , *Gluconobacter oxydans* or *Acetobacter aceti*

Symptoms

- Infected fruits do not show any external symptoms, even when fully ripe. Internally, the flesh may be water-soaked or light pink and have an aromatic odour, although these symptoms may not be obvious immediately. When sterilized by heat during canning, infected tissue darkens to colours ranging from pink to dark brown.
- In some fruits, only one or a few fruitlets may be infected. In highly translucent, low-brix fruit, the entire cylinder can be invaded.

Infection and spread

The bacteria infect through the open flower during cool weather. Disease incidence increases in dry conditions before flowering, followed by rainfall during flowering. The bacteria are thought to be carried by nectar feeding insects and mites to open flowers from infected, decaying fruit near flowering fields.

Pineapple fruit core affected by pink disease (left) compared with one not affected by the disease (right)

Management

This disease occurs only sporadically when fruits develop under cool, wet conditions. Since the bacteria are killed by high temperatures, pink disease occurs mainly in spring (September–October). The incidence of infected fruit is very low. Management is not usually warranted. Smooth Cayenne is relatively resistant.

■ VIRUS ASSOCIATED DISEASES

■ MEALYBUG WILT DISEASE

Pathogen

Mealy bug wilt disease is caused by ampelovirus transmitted by mealy bugs.

Symptoms

- The early symptoms are a slight reddening of leaves about halfway up the plant. The leaf colour then

Mealy bug wilt disease causes reddening and yellowing in leaves and dieback in the leaf tips

changes from red to pink and leaves lose rigidity, roll downwards at the margin and the tip of the leaf dies.

- The root tissue also collapses and the plant appears wilted. Plants can recover to produce symptomless leaves and fruit that are markedly smaller than fruit from healthy plants.
- Symptoms are most obvious in winter when plant growth and vigour are reduced.
- Disease development and incidence is affected by plant age at the onset of mealy bug infestation, with younger plants displaying symptoms two to three months following feeding, while older plants may take up to 12 months to develop symptoms.

Infection and spread

The disease is thought to be caused by viruses transmitted by mealy bugs with the pink mealy bug (*Dysmicoccus brevipes*) being the main vector. The disease is probably introduced in planting material that may not show obvious disease symptoms. Once established, the viruses are transmitted when the mealy bugs feed on young leaves. Mealy bugs are sedentary insects that are moved from plant to plant by attendant ants or by wind. Ants actively tend mealy bugs. The coastal brown ant (*Pheidole megacephala*) is common and active in pineapple plantations, but many other species can be involved in raising mealy bugs. Mealy bugs produce honeydew, which is harvested by ants for food. Ants also protect mealy bugs from predators and move them around and between plants. The removal of spiders from fields by ants often allows large populations of mealy bugs to develop, increasing the risk of severe mealy bug wilt outbreaks. The incidence is variable and sometimes high. The amount of wilt in a field is related to the number of mealy bugs present, the length of time they feed and the activity of ants.

Management

Use planting material from wilt-free areas or from fields with a low level of wilt disease. If less than 3% of plants show wilt symptoms, remove infected plants by hand and destroy them. Use recommended insecticides for mealy bug and ant control where more than 3% of plants show wilt symptoms. If more than 10% of plants show wilt symptoms, do not use the field as a source of planting material. Eradicate badly affected areas immediately after harvest. Keep headlands and field boundaries free from weeds and rubbish as these may act as reservoirs for ants and mealy bugs.

■ YELLOW SPOT

Pathogen

Tomato spotted wilt virus, Capsicum chlorosis virus (Tospoviruses)

Symptoms

- Infection occurs on young crowns when they are still on the fruit or during the first few months after planting. Small (2–5 mm), round, yellow spots appear on the upper surface of the leaves of young plants. These spots fuse and form yellow streaks in the leaf tissue, which soon become brown and die.
- The virus spreads to the leaves in the plant heart, causing the plant to bend sideways. Infection eventually kills the plant so that the virus is not transmitted to subsequent plantings. If the crown is infected while still on the fruit, the fruit dies from the top

downwards. Infections can occur through open blossoms causing the development of large, blackened cavities in the side of the fruit.

Infection and spread

The viruses are transmitted to pineapple plants by small flying insects (thrips). Infection occurs mostly on plants during early growth, and crowns on developing fruit are occasionally infected. As infection is always fatal, vegetative propagation does not spread the virus to subsequent plantings. Tospoviruses have a wide range of hosts among weed and crop plants. The disease is rarely seen.

Yellow spot symptoms on pineapple

Management

Keep the plantation free from weeds. Avoid destroying old weedy patches near young crown plantings or fields with developing fruit. If this is impossible, it may be necessary to first spray the old infected field to control thrips.

Registered/Suitable pesticides

Fungicides: Mancozeb (Indofil M-45 75WP, 3 g/l)
Carbendazim (Bavistin 50WP, 1 g/l)
Carbendazim 12WP+ Mancozeb 63WP (Saaf, 2 g/l)
Hexaconazole (Contaf 5SC, 2 ml/l; Samarth 2SC, 4 ml/l)
Nematicide: Carbosulfan 6G, 17 kg/ha, soil application
Insecticides: Chlorpyriphos (Hilban 20EC, 2.5 ml/l)
Imidacloprid (Tatamida 200SL, 0.3 ml/l)
Quinalphos (Ekalux 25EC, 2 ml/l)
Miticide: Dicofol 4 ml/l

Note: Use 500 l/ha for foliar spray and 1 l/m^2 for soil drenching

DISEASES OF PASSION FRUIT (*Passiflora edulis*)

Pathogen, Symptoms, Infection, Spread & Management

Joy P. P. & Sherin C. G., Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670
Muvattupuzha, Ernakulam, Kerala, India, Tel. & Fax: +914852260832, Email: prsvkm@gmail.com

Passion fruit (*Passiflora edulis*), a native of tropical America, belongs to Passifloraceae family which comprises of about 530 species. Among these, the 'yellow' passion fruit (*Passiflora edulis flavicarpa*), purple passion fruit (*Passiflora edulis*) and Giant variety (*Passiflora quadrangularis*) are widely cultivated in Kerala. The passion-fruit plant is a woody vine (climber) with very fast, vigorous, continuous and exuberant growth. Passion fruit grows well in tropical and subtropical regions, where the climate is hot and humid. Passion-fruit can be grown on a range of soils, sands to clay loams. Generally these vines are grown on deep, relatively fertile and well drained sandy clay soil. There are many factors contributing to reduction in longevity and productivity in passion fruit plants, especially diseases of viral, bacterial or fungal etiologies, among which passion fruit woodiness, bacterial spot, root and collar rot, fusarium wilt, anthracnose and scab are the most important.

FUNGI ASSOCIATED DISEASES

Fungal diseases affect passion fruit from seedling phase to adult plant stage harming roots, stems, leaves, flowers and fruits. During post harvest stage, several fungi affect plants in the field conditions resulting in great loss during the fruit storage, transport and commercialization. Diseases affecting the above ground part of the plant are anthracnose, scab, septoriosis and alternaria spot. Diseases caused by soil microorganisms are very difficult to control, especially fusarium wilt, collar rot and crown rot.

■ COLLAR ROT

Pathogen

Homothallic strains of *Haematonectria haematococca* and *Fusarium solani*

Symptoms

- First above ground symptom is the mild die back of the plant followed by changing of leaf colour to pale green. Wilting, defoliation and finally plant death occurs resulting from the complete necrotic girdling of the plant collar.

- Necrosis generally reaches 2 to 10 cm above ground and may migrate to roots. Tumescence and fissures in the affected collar bark show purple lesion borders, where reddish structures appear under high relative humidity.

- The disease generally affects plants one to two years after planting, although it may occur earlier in replanting areas where the pathogen has previously appeared. (Domsch *et.al.*1980, Nelson *et.al.*1983)

Infection and Spread

Haematonectria haematococca survives for years as chlamydospores in the soil and spreads by any practice resulting in the movement of infested soil. Infected seedlings are also responsible for spreading the pathogen. Wounding has got a profound effect on collar rot disease. The disease is known to interact with phytophthora rot, nematodes, ants and termite attacks. The disease is favored by high temperatures and relative humidity. Resistance to collar rot increases as plants age (Domsch *et.al*. 1980, Nelson *et.al.* 1983)

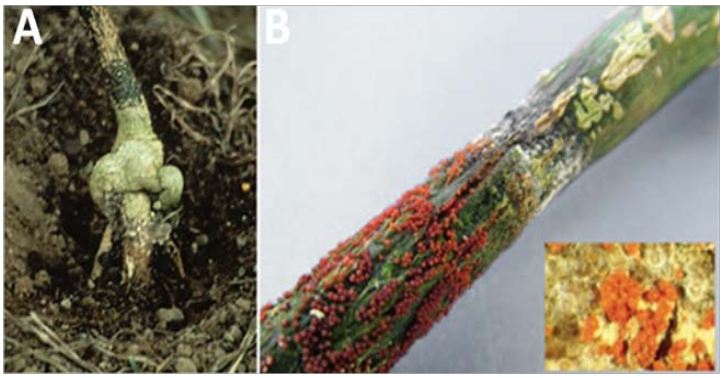


Figure 1 : (A)The swelling at the base of the vine is caused by *Fusarium solani* (B) Base rot on passion fruit showing white mycelium and crimson perithecia of *Haematonectria haematococca* .

Management

Areas previously infected with the disease should be avoided for new plantings and nurseries. Badly drained soils have to be avoided and careful irrigation has to be conducted in order to avoid excess water, water stress as well as injuries to plant collar and roots. Biweekly drenching of copper oxychloride reduce the number of plants developing collar disease. Under favorable conditions use of fungicides is ineffective. The use of a resistant root stock is an effective way to deal with the problem in the contaminated areas. (Domsch *et.al*.1980, Nelson *et.al.*1983)

FUSARIUM WILT

Pathogen

The fungus *Fusarium oxysporum*, which shows fast growing white pink, salmon or purple colonies in cultures with sparse to abundant aerial mycelium

Symptoms

- The glossy green leaves of young passion fruit plants show a pale green colour and mild die back. Drop of lower leaves, general plant wilting and sudden death take place as the disease progresses.
- In adult plants, the disease causes yellowing of young leaves, followed by plant wilt and death. Symptom development may be unilateral or encompasses the entire plant.
- The vascular system becomes darkened at the root, collar, stem and twig areas. The disease typically affects the xylem vascular system, leading to the impermeability of vascular walls and preventing the translocation of water to other plant parts.
- Under high relative humidity conditions, lesions and fissures can be found in the plant collar and stems. (Gardner 1989).

Infection and Spread

Resistant chlamydospores enable long term survival of the fungus in the soil. Germinated chlamydospores can infect the passion fruit plant triggering the spread. The fungus penetrates the roots and hypocotyls of plants mainly via injuries. The pathogen can spread systemically through mitochondria produced in infected vascular system and is passively transported by the transpiration flow. As the disease progresses the fungus may invade tissues adjacent to the xylem such as phloem and cortex, causing external cankers or stem fissures. Inside an orchard the fungus is spread by soil movements (machines, implements, shoes etc) and by run off or irrigation water. The disease intensity is greater in sandy soils and favored by high temperatures and relative humidity (Gardner 1989).

Management

Planting areas previously affected may be avoided. Use of healthy seedlings and careful control of weeds to avoid root injury can check the spread of disease. Another control measure that can be implemented is usage of resistant root stocks or resistant hybrids from crosses between purple and yellow passion fruits. (Gerlach and Nirenberg 1982, Nelson et.al. 1983)

Figure 2: Passionfruit vines infected with Fusarium wilt caused by *Fusarium oxysporum* f. sp. *passiflorae.*

ROOT AND CROWN ROT

Pathogen

Etiological agents are *Phytophthora cinnamomi* and *Phytophthora nicotianae*

Symptoms

- Phytophthora root and crown rot disease affects both adult as well as nursery plants. Mild cholorosis is followed by wilting, defoliation and death. Cortical tissues of the plants are exposed.
- Plant intumescence and bark fissures are found in the collar. Injured leaf shows a burned appearance. Occurrence of foliar blight followed by drop of flowers is observed.
- There is a change in leaf color from colorless to pale green, with leaves reaching a light copper colour. The affected plant shows burned –like black twig tips and flowers which eventually die. Large grayish- green aqueous spots can be viewed in fruits, which easily fall down. (Inch 1978)

Infection and Spread

The disease appears in specific spots and spreads from one plant to another. High disease incidence is observed in clay soils during rainy periods when temperatures vary between 26 - 30°C. Zoospores produced inside the sporangia and released in the presence of water are attracted

by root exudates. Reaching the root surface, the zoospores encyst and germinate, producing hyphae that colonize the intra and inter cells of the plant roots, destroying the external cortical tissue, reaching the cambium avoiding sap circulation. Chlamydospores and zoospores are resistant spores capable of surviving in soil and plant tissues for several months. Under favorable environmental conditions and in the presence of a host, chlamydospores and oospores can germinate and produce a great number of zoospores. Cardinal temperature for growth is 37°C. (Ploetz *et.al.* 2003)

Management

The elimination of diseased tissues during the initial stages of the disease and use of Bordeaux mixture can check the spread of disease. Applications of fungicides effective against oomycetous organisms directly applied on the plant collar soon after the beginning of the rainy season may control the disease. Pulverizations with copper oxychloride at an interval of every seven to ten days can control foliar blight. (Grech and Rijkenberg, 1991)

Figure 3: Crown Rot of Passion fruit caused by *Phytophthora nicotianae*

ANTHRACNOSE

Pathogen

Colletotrichum gloeosporioides is the causative agent of anthracnose

Symptoms

- Intense defoliation, twig wilt and fruit rot
- Spots, initially 2–3 mm in diameter and oily in appearance, are produced on the leaf. They become dark brown, round or irregularly shaped and 1 cm in diameter. The centers of spots become brittle and may break apart. Lesions also develop on petioles.
- As foliar lesions coalesce, large areas of the leaf die, resulting, eventually, in abscission. Dark brown spots, 4–6 mm in diameter, are produced on the branches and tendrils, eventually turning into cankers. Severe lesions can cause the death of shoots and a partial blighting of the plant
- Affected flowers abort, and immature fruit abscise. Lesions on fruit initially are superficial and light brown, and later become sunken and greyish to dark brown. They may be larger than 1 cm in diameter and may reach interior portions of the fruit. As fruit mature, the spots enlarge and become oily or light tan.
- The fruit skin becomes papery and acervuli are formed on lesions here and on leaves. Under high humidity, masses of red and orange spores form in acervuli. Dieback, characterized by reduced elongation of shoots, shortened internodes and an eventual wilting and death of these structures are the symptoms normally associated with anthracnose.

Infection and Spread

The disease is most observed in the second planting year. The fungus survives and sporulates in infected tissues and crop residues of passion flower. Fungal dissemination in the field is carried out by raindrops infected seeds, seedlings and cuttings. Long raining periods and average temperatures of 27°C are the ideal conditions for the occurrence of epidemics. During winter the incidence of disease is low. The incubation time observed in seedlings is six days. Host injury increase infection, but is not an obligate requirement. Quiescent infections occur on mature fruit where by infections stop development after apressorium formation. (Jeffries *et.al.*1990)

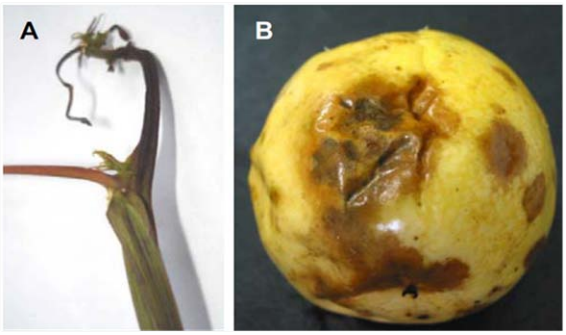


Figure 4 : (A) Death of Passion fruit shoots affected with anthracnose, (B) Fruit Rot due to anthracnose

Management

Use of pathogen free seedlings, pruning to eliminate affected areas, improved ventilation and light conditions help control the disease. Fruit should not be harvested during wet conditions, unduly exposed to sun light or kept for long in the absence of refrigeration. Pruning should be done when plants are dry and should be followed with applications of fungicides. Applications of mixed formulations of protective and curative fungicides are necessary during favorable conditions. Under intense rainy periods, fungicides have to be used weekly, while during scattered rain seasons fungicides have to be used in fifteen days interval. Applications can be suspended in dry seasons with no occurrence of dew. Fungicides coated as efficient against anthracnose are benzimidazole, cupric dithiocarbamate, chlorothalonil and tebuconazole. The fungicides prochloraz and imazazil show the best results for the control of post harvest rots. *Tricoderma* spp can control the disease in field or post harvest conditions. Thermal treatment of *Passiflora edulis* fruits at 42.5- 45 for eight minutes significantly reduces the disease incidence in fruits. (Phelps 1991)

■ SCAB

Pathogen

Scab, also known as Cladosporium rot is induced by *Cladosporium oxysporum* (Simmonds 1932).

Symptoms

- Plants infected with the Cladosporium show small round spots on the leaves. Spots are initially translucent, later become necrotic showing greenish-grey centers which correspond to fungal fructification.

- Lesions can perforate leaves, occur on veins and cause them to be deformed leading to abscission. Similar spots may appear on bud sepals or open flowers. High numbers of lesions on flower buds or on peduncles can greatly reduce the number of flower buds.
- Twigs and twig tips initially show lesions similar to the ones on leaves, which later turn into cankers of elongated and sunken aspect that become greenish – grey, where the pathogen fructification takes place. As scar tissue forms, branches become weakened and break in the wind.
- On small fruits, symptoms are slightly sunken with small dark circular spots. On bigger fruits lesions on fruit skin grow and become corklike, prominent and brownish. Lesions do not reach the inner fruit and consequently do not affect juice quality. Several lesions may form on the same fruit causing it to be deformed and stunted. (Manicom *et.al.* 2003)
- The disease mainly affects young tissues of leaves, branches, tendrils, flower buds and fruits, when not controlled cause significant damages. In field conditions it causes death of the twigs, can delay flowering and reduce the commercial quality of fruit.

Infection and Spread

Dissemination of the fungus occurs through infected seedlings, by wind and sprinkler water. High relative humidity promotes the infection with young tissues more susceptible to disease than adult. Incubation period is seven days in fruits and twelve days in leaves. Small necrotic spots appear on seedlings which show burn like symptoms after two weeks and eventually death occurs. The disease severity is high in spring time when temperatures are mild.

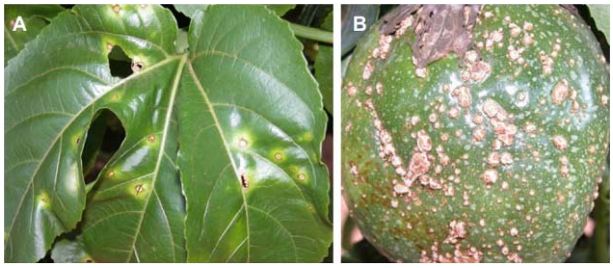


Figure 5: Scab Symptoms on Passion fruit (A) Leaf (B) Fruit

Management

High densities of seedlings and excessive irrigation are to be avoided in nurseries. Fungicide applications have to be periodically carried out. Adult plants should be provided with adequate ventilation. Pruning and cleaning of plants should be followed by incineration of infected tissues. Fungicide applications have to be carried out especially during periods of intense growth and flowering. Effective fungicides are tebuconazole, strobilurin, copper oxychloride, mancozeb, captan and chlorotalonil + copper oxychloride (Manicom *et.al.* 2003).

SEPTORIA BLOTCH (SPOT)

Pathogen

Three species of Septoria namely *Septoria fructigena*, *S. passifloricola* and *S. passiflorae* cause spot disease of which *S. passifloricola* seems to be more widely spread.

Symptoms

- Leaves are the most affected organs, showing light brown slightly round necrotic spots normally encircled by a chlorotic halo. A single lesion per leaf is sufficient to cause abscission, and even leaves without visible symptoms may fall prematurely.
- When the disease reaches 15-20% of leaves in the same plant, partial or even complete leaf abscission is observed. In young twigs, lesions may promote girdling leading to wilt and twig tips death.
- Lesions on flowers are similar to those on leaves. The primary infection in the calyx may reach the stalk, causing the early drop of flowers. The infection may occur at any stage of the development of the fruits, affecting maturation or development. Leaf and fruit abscission, twig wilt and plant death may occur under disease favoring conditions (Louw 1941).

Infection and Spread

S. passiflorae produces dark, spherical and sub-epidermic pycnidia in lesions. They may erupt and become ostiolate. The conidia are released in the hyaline cirri and are agglutinated by a mucilaginous substance. Conidia contained in the cirri are spread by water, dew and insects. The fungus survives in infected tissues, mucilage in the cirrus is thought to aid survival. Prolonged rains and mild temperature favor disease development. The optimum conditions for growth of fungus are temperature ranging from 5° to 35° C (Inch 1978, Manicom *et.al.* 2003, Trujillo *et.al.* 1994).

Management

Control measures used for the above ground diseases such as the use of carbamate and benzimidazole fungicides are generally enough to avoid damages caused by septoriosis in nurseries and field plants. Thiabendazole or thiophanate-methyl + chlorothalonil applied at 15 days interval is effective against this disease. Benomyl in a mixture or alternated with fungicides of different modes of action can be effective. (Peterson 1977, Louw 1941)

BROWN SPOT

Pathogen

Alternaria passiflorae and *A. alternata* are the causative agents

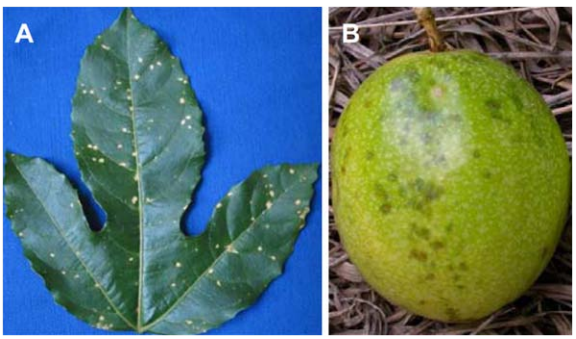


Figure 6 : Septoria blotch symptoms on passion fruit (A) Leaf (B) Fruit

Symptoms

- *Alternaria passiflorae* causes reddish brown spots on the leaves. Under high humidity, spots normally grow larger up to 2 cm in diameter become round and zonate.

- Spores can form a black thin mass covering the middle of the lesion, being more abundant on the abaxial surface. Abscission of the affected leaves occur rapidly causing intense defoliation.
- In twigs dark brown lesions are more elongated and may cause girdling and death of the terminal portion of these organs.
- Slightly circular spots occur on the mature fruits or when they are half way through their growth process. They are reddish brown, sunken affecting the pulp and damaging the commercial value.
- *A. alternata* causes smaller spots with chlorotic haloes on leaves and can induce defoliation. The stem lesions rarely kill vines. Spots on fruits have dark green and greasy margins.

Infection and Spread

The conidia are dispersed by wind, water and rain and occasionally by infected seedlings. The disease is more intense under high humidity and abundant rainfall, along with rising temperatures. The disease appears in fruits during the rainy season and disappears during the dry season. In young plants, after four days of inoculation first symptoms appear while typical symptoms appear ten days later. The pathogen survives in infected leaves, twigs and fruits in the plant and on the soil. Lesions are present in plants throughout the year, in sufficient numbers to ensure the continuity of the inoculum. (Brien 1940, Ram *et.al.*1977, Manicom *et.al.* 2003)

Figure 7: Passion fruit affected with brown spot disease

Management

Trimming vines to increase ventilation and penetration by fungicides can reduce disease pressure. The fungicides recommended are copper compounds, carbamates and strobilurins applied 7- 14 days intervals from the onset of symptoms and at greater intervals when conditions are less favorable. Under high humid conditions usage of mancozeb + iprodione are effective at controlling the disease. The use of more tolerant hybrids to *Alternaria spp*. applied with fungicides allowed better commercial fruit yield than the use of susceptible clones applied with fungicides (Hutton 1988, Nakasone *et.al.* 1975).

♦ VIRUS ASSOCIATED DISEASES

■ WOODINESS OF PASSION FRUIT

Pathogen

Passion fruit woodiness virus (PWV) and Cucumber woody virus (CWV)

Symptoms

- Infection causes a noticeable reduction in the development of plant. Leaves display severe mosaic, rugosity and distortion.
- Plants affected with PWV and CWV produce woody and deformed fruits. Severe mosaic, epinasty, defoliation and premature death of plants are associated with infection of PWV.
- Other common symptoms are leaf mottling and ring spot on the younger leaves. Fruits are symptom less or may show mild molting. Chlorotic spots on the leaves and dappled or faded fruits are often found

Infection and Spread

Viruses are normally transmitted by several species of aphids in a non persistent, non-circulative way. They can also be transmitted through grafting and experimental mechanical inoculation. Mechanical transmission by knifes, scissors and nails during cultural practices of trimming are observed. None of the viruses are found to be transmitted through seeds. Species of Passiflora when susceptible to this disease develop systemic infection, which may be symptomatic or latent (Chang 1992, Parry et.al. 2004).

Management

Chemical control of vectors is usually ineffective for the virus because of the non persistent relationship between the virus and aphid vectors. Specific recommended cultural practices can be followed for minimizing the woodiness of passion fruit. Usage of virus free seedlings of new plantings, eradication of old and abandoned orchards before starting new crops, care during trimming operations to eliminate mechanical transmission of viruses, avoiding leguminous plants which may harbor the virus near the orchard and rouging of diseased plants by means of systematic inspections during the first five months after transplanting can aid in checking the incidence and spread of potyvirus infection in passion fruit vineyards (Gioria et.al. 2000).

Figure 8: Passion fruit woodiness disease. Compare the affected, distorted fruit with the healthy, smooth fruit.

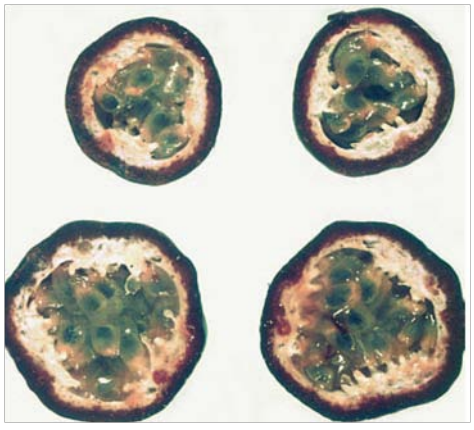


Figure 9 : Passion fruit woodiness disease. Cut fruit showing smaller cavity.

Figure 10 : Crinkled leaf symptoms of woodiness virus disease

Figure 11 : Molting of Passion fruit leaves caused by CWV

Pathogen

Gemini virus tentatively designated as Passiflora leaf mottle Virus

Symptoms

- Severe curling, distortion and mottling of leaves and fruits
- Reduced yields and fruit quality.
- Infected passion flower exhibits intense yellow mosaic of leaves and drastic reduction in the leaf lamina.
- The size of fruits per plant is small and deformed.

Infection and Spread

Virus is transmitted by white fly (*Bemisia tabaci*) from infected passion flower to bean and from the bean to bean but not from the infected bean to passion flower. Virus is not transmitted by sap inoculation or by seeds of infected plants.

Management

Usage of virus free seedlings of new plantings and eradication of old and abandoned orchards before starting new crops can check the spread of viral infection. Periodic rouging of diseased plants by means of systematic inspections can control the spread of disease. Chemical control of viruses is ineffective (Gioria et.al. 2000).

MOSAIC DISEASE

Pathogen

Passion fruit yellow mosaic virus (PaYMV)

Symptoms

- Infected plants exhibit a characteristic bright yellow mosaic, yellow net and leaf crinkle

Infection and Spread

The virus is not apparently transmitted by seeds. Diabrotics speciosa, a polyphagous beetle, found occasionally in passion flower plantations is the natural vector of PaYMV. (Crestani et.al. 1986)

Management

Usage of virus free seedlings of new plantings and eradication of old and abandoned orchards before starting new crops can check the spread of viral infection. Periodic roguing of diseased plants by means of systematic inspections can control the spread of disease. Chemical control of viruses is ineffective.

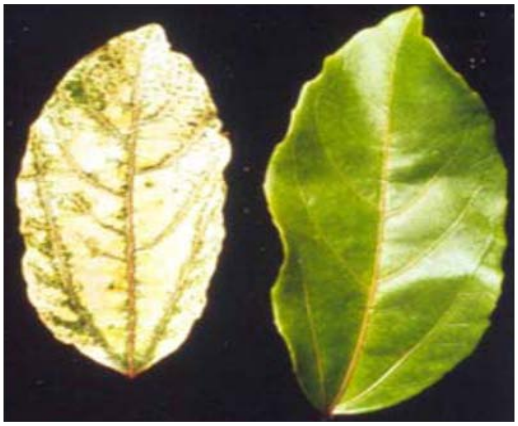


Figure 12 : Symptoms of passion fruit Yellow Mosaic Virus on affected leaf (left) Healthy leaf (right)

VEIN CLEARING

Pathogen

Passion fruit vein clearing virus of the Rhabdo virus family is the causal agent

Symptoms

Infected plants show clearing of the veins and reduced size of the leaves. Yield is severely affected and fruits are smaller in size

Infection and Spread

Virus is found in the perinuclear space of the cells. Infected plants show clearing of the veins and reduced size of the leaves. Yield is severely affected and fruits are smaller in size. Virus is transmitted during grafting but not while sap inoculation. Host range and vectors of this virus are unknown (Pares et.al. 1983).

Management

Usage of virus free seedlings of new plantings and eradication of old and abandoned orchards before starting new crops can check the spread of viral infection. Periodic roguing of diseased plants by means of systematic inspections can control the spread of disease. Chemical control of viruses is ineffective (Pares et.al. 1983).

Figure 13 : Vein clearing on passion fruit leaves caused by passion fruit vein clearing virus

♦ BACTERIA ASSOCIATED DISEASES

■ BACTERIAL SPOT

Pathogen

Xanthomonas axonopodis pv. *passiflorae*, an aerobic gram negative rod which forms bright yellow colonies in the culture medium is the causative agent.

Symptoms

- ♪ Diseased plants show well defined translucent, dark green anasarcous small spots encircled by a chlorotic halo on the leaves. Under favorable conditions, lesions become bigger and turn brown in colour affecting the entire leaf causing wilt and leaf fall.
- ♪ On latter stages infection spreads through leaf veins to reach the vascular system of the vines, causing longitudinal grooves, darkening of the vascular systems and portion dry. Transversal cut of infected vines exude bacterial pus. Incidence of this disease greatly reduces fruit production and eventually causes death of plant.
- ♪ Fruits are presented with dark or brownish green, anasarcous circular or irregular lesions with well defined edges. Bacterial exudates when dry form a hard crust over the lesions. These spots penetrate the pulp, causing fruits to fall before maturation or making fruits unmarketable (Fischer *et.al* 2008).

Infection and Spread

Infection occurs through natural openings or wounds followed by colonization of the pathogens in the inter-cell spaces and vascular tissues. Disease severity increases with high temperatures and relative humidity. Local dissemination of the bacterium is favored by wind, rain, irrigation and also through infected seedlings.

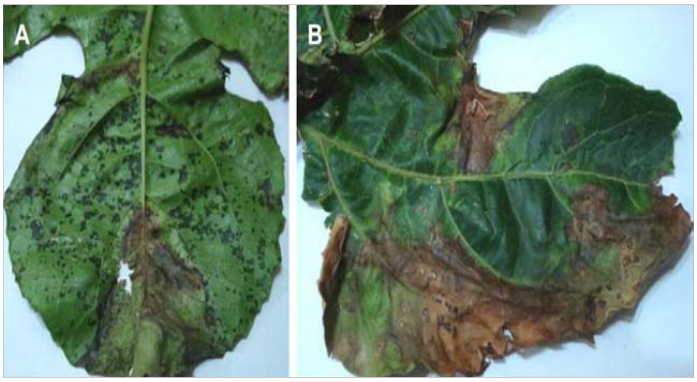


Figure 14 : Bacterial Spot caused by *Xanthomonas axonopodis* pv. *passiflorae* (A) Dark green and anasarcous lesions (B) Brown lesions

Management

Only preventive measures can be adopted as there are no effective chemical control measures available. Seeds and seedlings should be taken from healthy plants of disease free areas. Seed thermal therapy at 50℃ for 15 minutes is efficient to eliminate path ogen without affecting germination of seeds. New plantings should be done in the areas free from pathogens for at least two years. Use of wind breaks and adequate amount of fertilizers can keep the pathogens in distance. Avoid working on wet plants to prevent spreading of diseases. Use adequate amount of nitrogenous fertilizers, especially stimulates new shootings and delays maturation, making plants more susceptible to bacterium. The elimination of diseased parts of the plants and disinfection of

pruning tools and hands with bactericide products, such as those using quaternary ammonium and alcohol may reduce the spread of pathogen. Copper oxychloride and its mixture with mancozeb at 7 to 15 days interval decrease the intensity of disease. However under frequent rains and favorable environmental conditions of pathogens the use of cupric fungicides or streptomycin sulfate, highly soluble in water is washed away by rain. If there is no rain or no sprinkler irrigation, the product shows effective protection (Fischer *et.al* 2008).

BACTERIAL GREASE SPOT

Pathogen

Pseudomonas syringae pv. *passiflorae*

Symptoms

- This disease affects under-ripe fruits. Fruits develop small dark green areas, turning into golden to brownish greasy necrotic lesions.
- On a later stage a hard crust harboring several kinds of microorganisms covers the lesions. Leaves show severe necrotic lesions surrounded by a chlorotic halo.
- Shallow canker lesions can be observed on the vines directing to the death of the tip of the vines. (Baigent and Starr 1963, Bradbury 1986)

Infection and Spread

Penetration of the bacterium occurs most frequently via stomata and hydrathodes. Injury also contributes to the infection process. Infection is favoured by high relative humidity, a water film on the leaf surface and frequent rainfall. Local dissemination of the pathogen is enhanced by wind-blown rain and irrigation, and by workers handling wet plants, whereas long-distance dispersal occurs on seedlings. (Manicom *et.al.* 2003)

Management

Seeds and seedlings should be from healthy plants and, if possible, should be obtained from disease-free areas. Alternatively, seeds should be treated at 50°C for 15–30 min. Other complementary measures that should be adopted include: planting in areas that have not had the disease for the preceding 2 years; use of wind breaks; avoiding work on plants when they are wet; disinfesting pruning tools and hands; and using fertilizers judiciously, especially with respect to nitrogen. Chemical control is based on the use of mixtures of cupric and carbamate fungicides, or products that contain streptomycin or oxytetracycline. These measures have shown variable effectiveness that may be due to crop management, the quality and frequency of applications, the level of infection and susceptibility of the host plant, and virulence of the pathogen (Manicom *et.al.* 2003).

♦ NEMATODE ASSOCIATED DISEASES

■ ROOT – KNOTS AND CYSTS

Pathogen

Meloidogyne javanica is the causative agent

Symptoms

♪ Root system becomes deficient and weak with poor absorption of water and nutrients. Consequently plant shows lower growth and foliar yellowing with reduced productivity

Infection and Spread

Meloidogyne spp attacks the passion fruits by injecting the roots with certain toxic substances leading to the formation of root – knots and cysts.

Management

The use of healthy seedlings, crop rotation with plants that are poor hosts of nematodes, solarization, fallow and nematicides are recommended measures to control nematodes (Fischer *et.al* 2008).

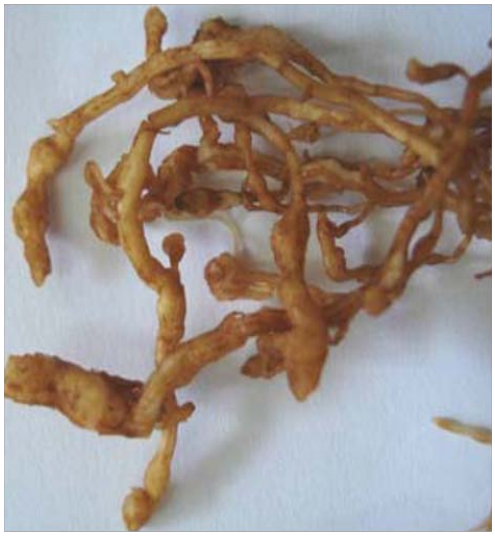


Figure 15: Root –Knot on yellow Passion fruit caused by *Meloidogyne javanica*

♦ PHYTOPLASMA ASSOCIATED DISEASES

■ OVERSHOOTING

Pathogen

Phytoplasma is the causative agent

Symptoms

♪ Chlorotic small leaves, shortening of internodes, excessive lateral shoots and abnormal flowers.

♪ There may be splitting and falling of fruits during their formation or just a reduction of their size.

Infection and Spread

The Phytoplasma which cause over shooting is a prokaryote without a cell wall which invades the phloem of the plants. It shows fast dissemination by vectors still unknown, sharp shooters are supposed to be involved mainly the one belonging to the Empoasca genus, which is often found in these crops. The pathogen may also spread through grafting (Fischer *et.al* 2008).

Figure 16 : Overshooting caused by phytoplasma

Management

To avoid introduction of phytoplasma into new production areas, it is necessary to carry out periodical inspection of plant nurseries and use healthy seedlings. Plants must be periodically inspected in the areas already infected by the disease and diseased plants have to be removed. It is known that phytoplasma infected plants treated with antibiotics belonging to the tetracycline group show a temporary reduction of symptoms (Bradel *et.al.* 2000).

REFERENCES

Baigent NL, Starr MP (1963) Bacterial Grease spot disease of passion fruit, *New Zealand Journal of Agricultural Research* 6, 24-38

Bradbury JF (1986) *Guide to Plant Pathogenic Bacteria*, CAB International, Wallingford, UK, 322 pp

Bradel BG, Preil W, Jeske H (2000) Remission of the free branching pattern of *Euphorbia pulcherrima* by tetracycline treatment. *Phytopathology* 148, 587-590

Brien RM (1940) Brown Spot (*Alternaria passiflorae* Simmonds). A Disease of the passion – vine in New Zealand. *New-Zealand Journal of Science and Technology*, Section A 21, 275-279.

Chang CA (1992) Characterization and comparison of passion fruit mottle virus, a newly recognized potyvirus, with passion fruit woodiness virus, *Phytopathology* 82, 1358-1363

Crestani OA, Kitajima, EW, Lin MT, Marinho VLA (1986) Passion fruit yellow mosaic virus , a new Tymovirus found in Brazil. *Phytopathology* 76, 951-955.

Domsch KH, Gams W, Anderson TH (1980) *Compendium of soil Fungi* (Vol 1), academic Press, Newyork, 120pp

Fischer Ivan H, Rezende Jorge AM (2008) Diseases of Passion Flower (*Passiflora* ssp.) *Pest Technology*, Global Science Books.

Gardner DE (1989) Pathogenicity of *Fusarium oxysporium* f.sp. *Passiflorae* to banana poka and other *Passiflora* spp. in Hawai. *Plant Disease* 73, 476-478

Gerlach W, Nirenberg H (1982) *The genus Fusarium- a Pictorial Atlas*, Paul Parey, Berlin, 355 pp

Gloria R, Espinha LM, Rezende JAM, Gasper JO, Kitajima EW (2002) Limited movement of Cucumber virus (CMV) in yellow passion flower in Brazil. *Plant Pathology* 51, 127-133.
Grech NM, Rijkenberg FHJ (1991) Laboratory and Field evaluation of the performance of Passiflora caerulia as a rootstock tolerant to certain fungal pathogens. *Journal of Horticultural Science* 66, 725-729.

Hutton DG (1988) The appearance of dicarboximide resistance in *Alternaria alternate* in passion fruit in south –east Queensland. *Australian Plant pathology* 17, 34-36

Inch AJ (1978) Passion fruit diseases. *Queensland Agricultural Journal* 104, 479-484.

Jeffries P, Dodd JC, Jeger MJ, Plumbley RA (1990) The biology and control of *Colletotrichum* species on tropical fruit crops, *Plant Pathology* 39, 343-366.

Louw AJ (1941) Studies on *Septoria passiflorae n.sp*. occurring on passion fruit with special reference to its parasitism and physiology. Scientific bulletin of the South African Department of agriculture 229, 51 pp

Manicom B, Ruggiero C, Ploetz RC, Goes A de (2003) Diseases of Passion fruit. In: Ploetz RC (Ed) *Diseases of Tropical fruit crops*, CAB international , Wallingford, pp 413- 441.

Nakasone HY, Aragaki M, Ito P (1975) Alternaria Brown spot tolerance in passion fruit. *Proceedings of the tropical Region of the American society of Horticultural Science* 17, 159- 169

Nelson PE, Toussoun TA, Marasas WO (1983) *Fusarium Species. An illustrated Guide for Identification*, Pennsylvania State University Press, 193 pp

Pares RD, Martin AB and Morrison W. (1983) Rhabdovirus –like particles in Passion fruit. *Australasian Plant Pathology* 12, 51-52.

Parry JN, Davis RI, Thomas JE (2004) Passiflora virus Y, a novel virus infecting Passiflora spp. in Australia and the Indonesian Province of Papua. *Australian Plant Pathology* 33, 423-427.

Peterson RA (1977) Benomyl resistance in *Septoria passiflora* Louw, *APPs Newsletter* 6, 3-4.

Phelps RH (1991) Identification and control of leaf and fruit disease of passion fruit at Orange groove. Technical Report,Caroni Research station 23, 78-80.

Ploetz RC, Lim TK, Menge JA, Rohrbach KG, Michailides TJ (2003) Common Pathogens of tropical fruit crops. In: Ploetz RC(ED) *Diseases of tropical Fruit crops*, CAB International. Wallingford, pp 413-441.

Ram B, Naidu R, Singh HP (1977) *Alternaria Macrospora* Zimm, a new record in passion fruit (*Passiflora edulis* Sims) from India. *Current Science* 46, 165.

Simmonds JH (1932) Powdery spot and fruit scab of passion vine. *Queensland agricultural Journal* 38, 143-152.

Trujillo EE, Norman DJ, Killgore EM (1994) Septoria leaf spot, a potential biological control for banana poka vine in forests of Hawaii. *Plant Disease* 78, 883-885.

INSECT PESTS OF PASSION FRUIT (*Passiflora edulis*): Hosts, Damage, Natural Enemies and Control

Joy P. P. & Sherin C. G., Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670 Muvattupuzha, Ernakulam, Kerala, India, Tel. & Fax: +914852260832, Email: prsvkm@gmail.com

Passion fruit is a vigorous perennial vine included in the Passifloraceae family. The most popular cultivated varieties are Yellow, Purple and Giant granadilla. The flowers are single and fragrant, 5-7.5 cm wide and borne at a node on the new growth. Fruits are dark-purple or yellow, rounded or egg shaped and contain numerous small, black wedge-shaped seeds that are individually surrounded by deep orange-colored sacs that contain the juice, the edible part of the fruit. Passion fruit develops well in tropical and subtropical regions, where the climate is hot and humid. Temperature, relative humidity, light intensity and precipitation have important influence on the longevity and the yield of the plants, but also favour the incidence of pests and diseases.

Passion fruit is attacked by several pest species of insects and mites that feed upon all parts of the plant. A limited number of species are clearly of major economic importance. Few have key pest status, while some species are secondary pests because they are sporadic or occur at low population levels and therefore do not require control strategies. Insect and mite pests that are frequently associated with passion fruit are described below, including their description, behavior, hosts, damage and control. (Santo, 1931; Lordello, 1952b; Correa et al., 1977; ICA, 1987; Dominguez-Gil et al., 1989; Figueiro, 1995; Lima and Veiga, 1995).

PRIMARY PESTS

Primary pests are those that can cause severe damage to the entire crop. Their occurrence will be in high numbers and proper control measures will have to be adopted to save the cultivars.

♦ LEPIDOPTEROUS DEFOLIATORS

Three heliconiine species, *Dione juno juno* Cramer, *Agraulis vanillae vanillae* Linnaeus and *Eueides isabella huebneri* Ménétries (Nymphalidae), are the most common lepidopterans feeding upon foliage of passion fruit (Dominguez-Gil and McPheron, 1992). *Dione juno juno* is the key pest which causes severe damage of the plant. *Juno* has orange wings with black borders and venation. The *A. vanillae* butterfly has red-orange wings, with black markings and venation, and silver spots on the underside. Two-thirds of the forewing of *Eueides isabella huebneri* is dark brown, almost black, with irregular yellow spots, and one-third is orange with black stripes. The hind wings are orange with black borders and a central stripe.

Fig 1: (A) Egg of Agraulis vanillae vanillae (B) Larvae (C) Adult Agraulis vanillae vanillae

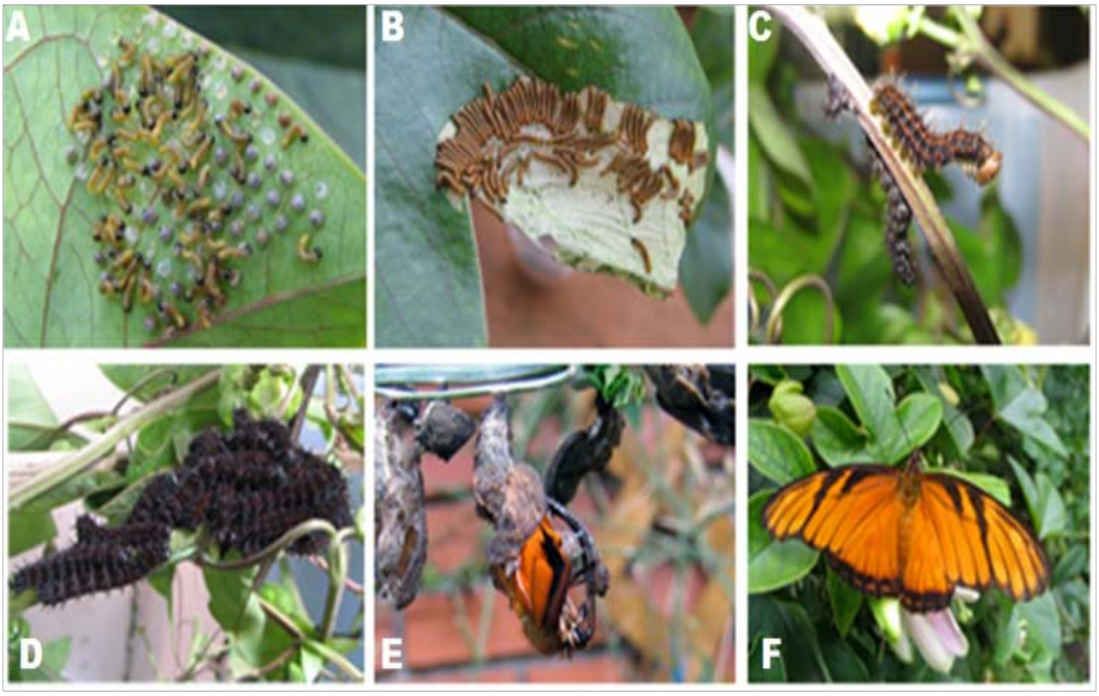


Fig 2: Life Cycle of *Dione juno juno Cramer*

Hosts

Caterpillars of *D. juno* feed on all Passiflora species, except *P. foetida* (Echeverri et al., 1991; Carter, 1992). According to Boiça Júnior et al. (1993), *P. alata, P. setacea* and the hybrid *P. alata* × *P. macrocarpa* are more resistant to attack by *D. juno* than *P. edulis, P. cincinnata, P. caerulea* and the hybrid *P. edulis* × *P. alata*.

Damage

Heliconiine defoliators reduce leaf area, thereby indirectly reducing yield. *Dione juno* usually causes damage that is more serious because of its gregarious behavior. Besides defoliation, the caterpillars may feed on the apical buds, flowers or stems (De Bortoli and Busoli, 1987).

Natural Enemies

Several predators and parasitoids have been reported for these heliconiids. However, these natural enemies are not considered to be effective.

Control

Control measures are crop inspection which includes hand picking and destruction of eggs and cater-pillars (Rossetto et al., 1974). On the other hand, these methods require considerable time and labour and are often impractical for a large-scale cultivation. In this case, injurious populations of defoliating caterpillars infesting passion fruit must be controlled with insecticidal sprays. Action thresholds have not been defined. Growers spray the foliage, often starting with appearance of the pest, and continue at regular intervals until the crop is harvested. In passion fruit it is very important to protect pollinating insects by timing insecticidal treatments when pollinators are not present in the field. Choosing an insecticide that is selective for the pest and less toxic to pollinators, predators and parasitoids is important in these agro-ecosystems. (Rossetto et al., 1974).

♦ COREID BUGS

In passion fruit producing areas, three main species of coreids are reported: *Diactor bilineatus Fabricius, Leptoglossus* spp. and *Holhymenia* spp. *D. bilineatus* is the most common species, and is known as the passion fruit bug because it feeds only on fruit of *passiflora spp.* Among the *Holhymenia*, *H. clavigera* (Herbst.) and *H. histrio* (Fabricius) are the most common species attacking passion fruit. The bugs *Leptoglossus*, *L. gonagra* Fabricius and *L. australis* Fabricius, usually cause damage to passion fruit. *D. bilineatus* are orange on the ventral face of the head, and the dorsal face is dark metallic green with two orange longitudinal lines that continue on the prothoracic tergum and the scutellum, both of which are dark metallic green. The adult body of *Holhymenia spp.* is black with orange spots. The legs are reddish orange. The head, the prothoracic tergum and the scutellum are black with white spots (De Bortoli and Busoli, 1987; Brandão et al., 1991; Dominguez-Gil, 1998).

Fig 3: *Gonagra Fabricius*

Fig 4: *L. australis* Fabricius

Fig 5: *Leptoglossus phyllopus*

Hosts

Besides passion fruit, *H. clavigera* feed on guava (Fancelli and Mesquita, 1998). *L. gonagra* feeds on a large number of host plants, including passion fruit, chayote, citrus, tobacco, guava, sunflower, cucumber, grape, pomegranate, São Caetano melon (*Cayaponia espelina*), bixa (*Bixa orellana*), araçazeiro (*Psidium araca*) and *Anisosperma passiflora* (Chiavegato, 1963).

Damage

Passion vine bugs migrate from surrounding scrub to infest passion fruit plantations. Neglect of vines may allow populations of the bug to build up. Feeding usually occurs on flowers or green-mature fruit. Nymphs often cluster on fruit when feeding. Damage to mature fruit is not pronounced; however, young fruit develops dimple-like surface blemishes at the feeding sites (Murray, 1976). Both immature and adult bugs injure the crop, piercing stems, leaves, fruits and flowering buds, by sucking plant juices. However, the nymphs prefer to feed on flowering buds and young fruits, usually resulting in excessive dropping. The adults may also attack leaves, stems and fruits at any stage of ripening. If larger fruits are fed upon, they wilt and show a wrinkled surface. *Leptoglossus gonagra* often causes misshaping or dropping of young fruits (Chiavegato, 1963). In small passion fruit producing areas, hand picking and destruction of eggs, nymphs and adults is recommended (Mariconi, 1952).

Natural Enemies

Natural enemies are present for many of the passion vine bugs. *D. bilineatus* eggs are parasitized by *Hadronotus barbiellinii* Lima (Scelionidae). Eggs of *H. clavigera* are reported to be eaten up by *Hexacladia smithii* Ashmead (Encyrtidae) (Silva *et al*., 1968).

Control

Removal of the alternate cucurbit host, 'São Caetano melon', a preferred host of *L. gonagra*, and avoiding the cultivation of chayote and *Anisosperma passiflora* in adjacent areas can reduce pest densities (Chiavegato, 1963). Regular inspection during the summer months aids to detect any build-up of *L. australis* (Murray, 1976).

♦ STEM WEEVIL

The stem weevil, (*Philonis spp*.) is included in the Curculionidae family. They are nocturnal. Adults of P. *passiflorae* are about 7 mm in length, brown with whitish elytra with two brown stripes. Adults of *P. crucifer* are 4 mm in length, brown with black markings.

Fig 6: Stem weevil (*Philonis* sp

Hosts

Yellow passion fruit is susceptible to attack by *Philonis* spp. while *Passiflora alata*, *P. maliformis*, *P. serrato digitada* and *P. caerulea* are not infested by this pest (Oliveira and Busoli, 1983). Cruz *et al*. (1993) observed that yellow passion fruit is very much susceptible to *Philonis obesus* attack, but *P. alata* and *P. giberti* show some plant resistance.

Damage

Larvae of *Philonis* spp. feed within the stems, opening longitudinal galleries inside stems that prevent plant development. The attacked stems are easily identified by the presence of excrement and sawdust (Santos and Costa, 1983). As the larva develops, infested stems become weak, frail and die (Fancelli, 1992a). Simultaneous attack of several larvae is characteristic of weevil infestations, which causes hypertrophy in stems where the pupal cell will be constructed (Rossetto *et al*., 1978; Oliveira and Busoli, 1983; Racca Filho *et al*., 1993). Attack by the stem weevil also causes fruit drop before maturation (Costa *et al*., 1979).

Control

Periodic inspection of the crop is essential for an early detection of weevil-infested stems (Fancelli, 1992a). When infestation symptoms are detected on the crop, affected stems should be pruned and burned (De Bortoli and Busoli, 1987). According to Leão (1980) and Costa *et al*. (1979), a contact insecticide (e.g. decamethrin at 25% (5–10 g a.i. ha−1)) should be applied during early afternoo n hours for stem weevil control, at the time of adult emergence. After 4–5 days, systemic insecticides for control of future stem infestations should be used.

♦ FLIES

Anastrepha Schiner (Tephritidae) and *Lonchaea* Fallén (Lonchaeidae), *A. consobrina* are the common genera of flies damaging passion vines. *A. curitis* Stone, *A. dissimilis* Stone, *A. fraterculus* (Wiedmann), *A. kuhlmanni* Lima, *A. lutzi* Lima, *A. pseudoparallela* (Loew), *A. striata* Schiner, and *A. xanthochaeta* Hendel are the most common species associated with passion fruit (Santos and Costa, 1983; Teixeira, 1994; Zucchi, 1988, 2000). *Anastrepha pallidipennis* (Chacón and Rojas, 1984), the oriental fruit fly, *Bactrocera dorsalis* (Hendel), melon fly, *Bactrocera cucurbitae* Coquillett and the Mediterranean fruit fly, *Ceratitis capitata* Wiedmann, are known to attack the passion fruit vines in Hawaii, USA (Back and Pemberton, 1918); however, the relative importance of each species appears to vary with respect to location of the vineyard (Akamine *et al*., 1954).

Fig 7: *Lonchaea fallén*

Anastrepha adults are 6.5–8.0 mm in length, predominantly yellow in colour, with brown and yellow markings on the wings. The adult Medfly is a smaller colorful insect with yellow and black

markings on the body and black and orange markings on the wings. The adult of *Bactrocera tryoni* is wasp-like in appearance, about the size of a house fly, with transparent wings bearing a dark band on the front margin. Bright yellow patches interrupt the general reddish brown body colour. The adult *Dasiops curubae* is blackish blue. The wings are hyaline and slightly smoky yellowish, while the calypters and wing fringes are pale yellowish (Steyskal, 1980). The adult *Dasiops inedulis* is bright metallic dark blue with hyaline wings; the calypters and wing fringes are yellowish to nearly white (Steyskal, 1980).

Hosts

The highly polyphagous *Anastrepha* spp. infest approximately 270 plant species and are considered to be the major fruit pests of tropical and subtropical America. Passiflora act as host for the larvae of two groups of *Anastrepha* namely *chiclayae and seudoparallela.* (Norrbom and Kim, 1988; Stefani and Morgante, 1996). Larvae of *A. limae* Stone feed upon fruits of *P. quadrangularis* (Stone, 1942; Caraballo, 1981). Lordello (1954) observed infestations by *Anastrepha* and *Lonchaea* species on *Passiflora quadrangularis* and *P. macrocarpa*. *Dasiops inedulis* is reported to be a serious pest of purple granadilla, *P. edulis* (Steyskal, 1980). This species has been implicated in 21–65% loss of flowering buds of passion fruit in the Cauca Valley (Colombia) (Peñaranda et al., 1986). *Dasiops passifloris* attacks fruits of *P. suberosa* (Steykal, 1980).

Damage

Fly species feed upon the fruits of Passiflora spp., and also attack flowering buds. *Neosilba pendula* and *Dasiops* sp. (Lonchaeidae) are the most common species attacking flowering buds of passion fruit (Rossetto *et al*., 1974; Silva, 1982; Fancelli and Mesquita, 1998). Other flies such as *Lonchaea cristula* McAlpine (Lonchaeidae) and *Zapriothrica salebrosa* Wheeler (Drosophilidae) may also feed upon flowering buds (Chacón and Rojas, 1984).

Fruit fly adult damage is caused by oviposition in green fruits, causing disfigurations of the fruit surface. The larvae damage the fruit by feeding on its pulp, contaminating it with bacteria and fungi and causing premature fruit drop (Medina *et al*., 1980; Santos and Costa, 1983; Morgante, 1991). The oriental, melon, and Mediterranean fruit flies puncture the fruit while the rind is still tender (Akamine *et al*., 1954). As the fruit enlarges, a woody area (callus) develops around the puncture. If the fruit is small and undeveloped, the damage may be sufficient to cause it to shrivel and fall from the plant. If the fruit is well developed, it may continue to maturity. At the time of ripening, the area around the puncture has the appearance of a small, woody crater, which disfigures the outer appearance of the fruit, but does not impair pulp quality. Although oviposition scars are present on ripening fruits, they generally do not contain living larvae. Larvae appear to be able to develop better in immature than in mature fruit. Oviposition by *B. tryoni* in immature green fruit also results in the formation of calluses in the skin of the fruit at the puncture site. Punctured fruits may persist on the plant to maturity but are not acceptable for fresh market sale because of the damage (May, 1953; Hargreaves, 1979).Passion fruit increase rapidly in size during the first 10–15 days after fruit set. During this period the skin of the fruit is turgid and easily punctured by the ovipositor. Infested immature fruit shows characteristic skin blemishes. The woody tissue, which forms around the eggs, develops a hard raised area around the puncture mark. Egg laying or puncture often causes young fruit to shrivel and drop. Puncture marks are difficult to detect on ripe fruit. A few days after larval infestation, mature fruit will show wrinkling and breakdown.

Natural Enemies

Natural enemies of fly species are larval parasitoids. *Doryctobracon enderlein*, *Diachasmimorpha viereck*, *Opius wesmael*, *Psyttalia walker* and *Utetes foerster* are the most common larval parasitoids of tephritid fruit flies (Wharton, 1996). *Pachycrepoideus vindemiae* (Rondani) and *Spalangia endius* walker (Pteromalidae) are pupal parasitoids of Medfly (Back and Pemberton, 1918). Larvae of *N. pendula* are parasitized by *Alysia lonchaeae* Lima, *Ganaspis carvalhoi* Dettmer, *Tropideucoila weldi* Lima (Cynipidae), and *Opius* sp. and preyed upon by *Belonuchus rufipennis. (*Silva *et al*. (1968).

Control

One of the most important steps in controlling fruit flies is the elimination of over-ripe fruits in which the flies breed and on which the adults feed. Santos and Costa (1983) recommended that passion fruit must be planted far away from coffee plantations and wild host plants that grow adjacent to the passion fruit crop should be removed. Fruit flies may be controlled using bait sprays composed of molasses (7%) or protein hydrolysate (1%) and an insecticide. The bait is sprayed over 1 m^2 of the plant canopy, using 100–200 ml of bait per plant (Santos and Costa, 1983). The bait should be applied during the night (Rossetto *et al*., 1974). Bud flies may be controlled by insecticide baits composed of fenthion, molasses and water. (Boaretto *et al*., 1994) The bait is applied at the beginning of the flowering peak, and usually three applications spaced at 8–10 days are necessary.

♦ MITES

Brevipalpus phoenicis (Geijskes) (Tenuipalpidae), the red spider mites *Tetranychus mexicanus* (McGregor) and *T. desertorum* Banks (Tetranychidae) are known to infest passion fruit plants. Warm temperature and low precipitation favour development of these species (Haddad and Millán, 1975; Oliveira, 1987; Brandão *et al*., 1991) *Polyphagotarsonemus latus* (Tarsonemidae) prefers high temperatures and greater than 80% relative humidity (Oliveira, 1987; Brandão *et al*., 1991)

Hosts

Brevipalpus phoenicis feeds on citrus, coffee, cashew, papaya, banana, guava, pomegranate, apple, loquat, peach, pear, grape, grevillea, and various weeds (Oliveira, 1987). *Tetranychus desertorum* occurs on cotton, sweet potato, bean, papaya, passion fruit, strawberry, peach, tomato, grape, and certain ornamentals. *Tetranychus mexicanus* feeds upon cotton, citrus, apple, papaya, passion fruit, pear, peach, cacao, walnut, and ornamentals (Flechtmann, 1989). Hosts of *P. latus* are bean, potato, cotton, coffee, citrus, apple, pumpkin, walnut, grape, peach, pepper, rubber plantation, and various weeds (Oliveira, 1987).

Damage

Brevipalpus phoenicis is responsible for general discoloration of the leaves, and necrosis, culminating in leaf drop. Attacked young stems dry from the extremity to the base and eventually die (Flechtmann, 1989). *B. papayensis*, known as red mite, is one of the most troublesome pests of passion fruit, but it is usually most damaging in areas of low rainfall and during prolonged dry weather. Passion fruit vines display yellowing, shriveling, and falling of the leaves. With heavy and prolonged infestation, leaf fall increases and the vine has the appearance of dying back. At the same time, developing fruit may begin to shrivel and fall prematurely from the plant. Close examination reveals the presence of mites as scattered reddish patches on the surface of the fruit, particularly around the stem end, along the midrib and veins of the leaf, especially on the under-surface. If red spider mites are left uncontrolled, the plant may eventually die (Akamine *et al*., 1954). Red spider mites cause a general weakening of the plants. Initial damage to foliage appears as fine silver speckling on the lower surface of the leaves, which turn brownish on the upper side as mites continue to feed. If large numbers of mites are present, entire leaves or plants turn yellow. (Oliveira, 1987). Photosynthesis and transpiration of the plants are suppressed. Dense populations of spider mites produce silken webs that cover the leaves. Heavy infestations cause leaves to drop and plants to lose vigor (Oliveira, 1987). *P. latus* induces malformations in developing leaves, which later dry and drop. It may attack flowering buds, causing a reduction in the number of flowers, and in turn, of fruits produced per plant (Oliveira, 1987; Flechtmann, 1989).

Natural Enemies

Important natural enemies of spider mites are predacious mites belonging to Phytoseiidae. The life history of these predators is closely related to that of their host. Larvae and adults of *Stethorus* sp. (Coccinellidae) were also observed as predators of *T. mexicanus* in passion fruit plantations.

Control

Periodic inspections of the orchard and other adjacent hosts, including weeds, are essential to verify the occurrence and first symptoms of mite attacks (Oliveira, 1987; Brandão *et al*., 1991). Selective miticides, dosages, timing, and refining application techniques may be useful in an integrated mite management system. The four principal requirements for a practical operation are: (i) presence of predacious mites in the orchard; (ii) knowledge of the appearance and habits of plant feeding and predacious mites; (iii) careful examination of relative numbers of predators and plant-feeding mites, particularly during a period when rapid population changes are occurring; and (iv) knowledge of pesticides to use, how to use them, and what pesticides to avoid, in order to conserve predators. Fenthion, propargite, chlorfentezine, and avermectin are effective miticides.

SECONDARY PESTS

Secondary pests include various species of insects that may become abundant, and occasionally damage the passion fruit crop. The insects in this group are either associated frequently with a particular environmental condition or else occur within limited geographical areas.

♦ APHIDS

Aphids (Aphidae) are known to attack passion fruit vines, although they seldom cause serious damage. Three species of aphids, *Myzus persicae* (Sulzer), *Aphis gossypii* (Glover), and *Macrosiphum solanifolii* Ashmead (*M. euphorbiae)* must be regarded as potentially important pests of passion fruit.

Hosts

Peach is the preferred primary host of *M. persicae*. It may infest other Prunus species, in particular almond and plum. Its secondary host plants include numerous wild and cultivated plants, such as passion fruit (Barbagallo et al., 1997). *Aphis gossypii* infests numerous species of dicotyledonous plants, including passion fruit. Favoured hosts are in the *Malvaceae* (cotton, hibiscus, etc.) and Cucurbitaceae (pumpkin, cucumber, watermelon, melon) (Barbagallo et al., 1997). M. solanifolii is a very polyphagous species, showing preference for the Solanaceae, i.e. potato, tomato, etc. (Barbagallo et al., 1997).

Damage

Aphids cause malformation in foliage, and they are more important as disease vectors. *Myzus persicae* and *A. gossypii* transmit virus disease that causes hardening of fruits. (Brandão *et al*., 1991; Piza Júnior and Resende, 1993). *Myzus persicae* and *M. solanifolii* are vectors of the passion fruit woodiness virus.

Natural Enemies

Naturally occurring predators and parasites are effective against aphids. The Coccinellidae are effective against cotton aphids and in particular the larval stage of *Scymnus*. Other predators include the Chrysopidae (*Chrysoperla*), Cecidomyiidae (*Aphidoletes*) and Syrphidae (*Syrphus*). Parasitism by *Lysiphlebus* sp. (Aphidiidae) has been reported (Barbagallo *et al*., 1997). According to Grasswitz and Paine (1993), *Lysiphlebus testaceipes* (Cresson) parasitizes *Myzus*, *Aphis*, and *Macrosiphum*. Silva *et al*. (1968) reported parasitism of *M. solanifolii* by *Aphidius platensis* Brèthes, *A. brasiliensis* Brèthes, *Diaeretiella rapae* (McIntosh) (Aphidiidae), and predation by *Bacha clavata* (F.) (Syrphidae), *Coccinella ancoralis* Germar, *Cycloneda sanguinea* (L.) and *Eriopis connexa* (Germar) (Coccinellidae).

Control

Proper use of insecticides and avoidance of host plantations near to the passion fruit wine yards can control the attack of aphids in passion crop cultivars.

♦ CATERPILLARS

Caterpillars of *Azamora penicillana* (Walker) (Pyralidae) are defoliators of passion fruit (Santos and Costa, 1983; Fancelli, 1992b; Fancelli, 1993). *Peridroma saucia* (Hübner) (Noctuidae) attacks the floral structure and may reduce fruit production (Chacón and Rojas, 1981). *Pyrausta perelegans* (Hampson) (Pyralidae) is also associated with passion fruit flowers. Caterpillars of *Aepytus* (*Pseudodalaca*) *serta* (Schaus) (Hepialidae) and *Odonna passiflorae* Clarke (Oecophoridae) are passion fruit stem borers (Chacón and Rojas, 1984).

Host

A. penicillana was reported damaging a wild species of passion fruit (*Passiflora cincinnata*) (Fancelli, 1993) *P. saucia* damages and causes reduction in fruit production of curuba (*Passiflora mollissima*). It is a polyphagous insect, feeding on potato (*Solanum tuberosum*), oak (*Quercus suber*), *Calendula officinallis*, cotton, tobacco, bean, tomato, lucerne, soybean, and beet (Chacón and Rojas, 1981).

Damage

Caterpillers of A. penicillana cause defoliation, the most serious damage is caused by the phytotoxic effects of the fluid secreted by the caterpillar on the leaves and young stems. Heavy infestations cause leaves to dry and drop, and passion fruit plants lose vigour and bear fewer flowers. In Bahia, Brazil, the population peak of this pest occurs during the rainy season (April to June) (Santos and Costa, 1983; Fancelli, 1992b, 1996). *P. saucia* larvae feed upon floral structures of *P. mollissima*. Young larvae migrate from leaves to the flowers where they feed on the floral tube, nectary and gynophore, causing flower dropping. The sixth instar larvae may occasionally continue feeding on the young fruit, or drop onto the soil to pupate. In Colombia, *P. saucia* infested 64% of the flowers during the summer (July to September) (Chacón and Rojas, 1981). Larvae of *A. serta* bore into roots located near the surface, and occasionally bore into stems. Stem injury is characterized by the presence of sawdust. A single larva is regularly found in 1-year-old plants, while in 6–8-year-old plants, up to five larvae may develop (Chacón and Rojas, 1984). The damage of *O. passiflorae* caterpillars is characterized by the presence of sawdust outside the principal and lateral stems. Several larvae in different stages of development attack simultaneously at the same point of the stem, and cause cellular hypertrophy. They form galleries in different directions, resulting in total destruction of the stem. The caterpillars of *P. perelegans* infest 6-month-old plants and remain during the whole vegetative period. They attack the buds and developing flowers, feeding on nectaries, gynophores, and young fruits (Chacón and Rojas 1984).

Natural Enemies

Natural predators are effective against *P. saucia*. A tachinid fly, *Incamyia* sp., is an important factor for reducing the population of *P. saucia* caterpillars. Another dipterous parasitoid is *Megaselia scalaris* (Phoridae). Adults of the predator *Anisotarus* sp. (Carabidae) feed on caterpillars and prepupae. . The larval stage of *O. passiflorae* is infected with the fungus *Beauveria bassiana* and is parasitized by the hymenopteran, *Neotheronia* sp. (Ichneumonidae), *Sathon* sp. (Braconidae) and *Enytus* sp. (Ichneumonidae) parasitize larvae of *P. perelegans*.

Control

The infestation of *A. serta* depends on the wood used to make the trellises. Use of resistant wood such as mangrove (*Rhizophora mangle*) can check the infection of caterpillars. Wood of Barbados cherry (*Malpighia glabra*) and *Cassia tomentosa* are susceptible to attack by *A. serta* and are not recommended for trellises.

♦ MEALY BUGS

Citrus mealy bug, *Planococcus citri* Risso, and the passion vine mealy bug, *Planococcus pacificus* Cox (Pseudococcidae), are pests of lesser importance on passion fruit. Citrus mealy bug, *P. citri*, is a small, oval-shaped sucking insect commonly found on passion fruit. Mealy bugs characteristically aggregate on the plant, especially at leaf nodes and under dead leaves and trash. Aggregation may also occur under dried flower bracts. Secretion of a sugary solution from the mealy bugs promotes growth of a black fungal mould on the fruits and leaves. Ants are often found tending mealy bugs for this secretion and interfere with the natural control of the Mealy bugs by parasites and predators.

Fig 8: Mealy Bug on Passion fruit leaf

Damage

If a severe infestation occurs, loss of vigour, leaf drop, and fruit malformation may occur. Unchecked, an infestation may cause death of the plant (Murray, 1976; Swaine *et al*., 1985).

Natural Enemies

Lady beetles (Coccinellidae), especially mealy bug lady beetle, *Cryptolaemus montrouzieri* Mulsant and maculate lady beetle, *Harmonia octomaculata* (Fabricius), substantially reduce mealy bug numbers. Of secondary importance are small wasp parasitoids such as *Leptomastidea abnormis* (Girault) (Encyrtidae) and *Ophelosia* sp. and lacewing larvae (*Oligochrysa lutea* (Walker)) (Murray, 1978; Swaine *et al*., 1985). *P. citri* is parasitized by *Apanteles para guayensis* Brèthes (Braconidae), *Coccophagus caridei* (Brèthes) (Aphelinidae), *Anagyrus coccidivorus* Dozier, *A. pseudococci* (Girault), *Leptomastidea abnormis* (Girault), *Leptomatrix dactylopii* Howard (Encyrtidae) and *Pachyneu ron* sp. (Pteromalidae). *Leptomastix dactylopii* is commercially available. It is a yellowish brown wasp that lays its eggs in late instar nymphs and adult Mealy bugs. *Leptomastix* prefers hosts in warm, sunny, humid environments. It may complete one generation in 2 weeks at 30˚C or in 1 month at 21˚C (Fisher, 1963).

Control

Clusters of mealy bug on dead leaves are well protected from the insecticide sprays, and little control can be achieved unless vines are cleaned thoroughly to allow spray penetration. Pruning may enhance the effectiveness of the spray; however, this is often impractical, as laterals to be pruned are generally bearing fruits (Murray, 1976). According to Murray (1976), occasional outbreaks of this pest are best controlled by two sprays of 1 : 60 Neem oil or methidathion 0.05% combined with 1 : 100 Neem oil, 2 weeks to 1 month apart. Oil in the ratio 1:60 is preferred, as methidathion is highly toxic to the mealy bug's natural enemies. For good control, thorough coverage is essential.

♦ SCALES

Soft brown scale (*Coccus hesperidum* Linnaeus) (Coccidae) may occasionally infest leaves and stems of passion fruit. California red scale, *Aonidiella aurantii* (Maskell) (Diaspididae) is most common on older passion fruit vines (Swaine *et al*., 1985).

Damage

Soft scales and diaspidids injure plants by sucking sap, and when in numerous can kill the plant. They sometimes heavily encrust the leaves, fruits, twigs or branches. Mealy bugs may be found on almost any part of the host plant from which they suck the sap (Murray, 1976; Swaine *et al*., 1985).

Natural Enemies

Parasitic wasps are important to control *A. aurantii*, mainly *Comperiella bifasciata* (Howard) and *Aphytis chrysomphali* (Mercet) (Aphelinidae). (Murray, 1976; Swaine *et al*.,1985). *Azya luteipes* Mulsant, *Coccidophilus citricola* Brèthes, and *Pentilia egena* Mulsant have been recorded as predators of California red scale. Two species of pathogenic fungi of California red scale are *Nectria coccophila* and *Myriangium duriaei* (Silva *et al*., 1968). According to Forster *et al*. (1995), *Aphytis melinus* is the most important parasitoid attacking California red scale. The female *A. melinus* feeds on and oviposits in immature scales, preferring the virgin adult female scale. The solitary, ectoparasitic larva leaves a flat and dehydrated scale body beneath the scale cover, where the parasitoid's cast skin and faecal pellets (meconia) may be observed. The parasitoid's short life cycle (10–20 days) results in two or three parasitoid generations for each scale generation. *Comperiella bifasciata* is an important encyrtid that parasitizes California red scale. Adult parasitoids are black, with two white stripes on the female's head. One parasitoid generation requires about 3–6 weeks to develop, with faster development occurring on larger (later instar) hosts and at warmer temperatures. Parasitoids of *C. hesperidum* in Argentina are *Aneristus coccidis* Blanchard, *Coccophagus caridei*, *Ablerus ciliatus* De Santis (secondary parasitoid) (Aphenilidae), *Aphycus flavus* Howard, *A. luteolus* (Timberlake) and *Cheiloneurus longisetaceus* De Santis (Encyrtidae). Among the predators is *Azya luteipes* Mulsant (Coccinellidae) (Silva *et al*., 1968).

Control

Chemical control is often not required since parasitization by small wasps substantially reduces populations. For effective chemical control, a 1: 60 Neem oil spray is satisfactory (Murray, 1976).

♦ TERMITES

Termites are increasingly common in passion fruit plantations. Three termite species, *Heterotermes convexinotatus* (Snyder), *Amitermes foreli* Wasmann, and *Microcerotermes arboreus* Emerson are observed to feed on roots and stems of 2–4-year-old passion plants.

Hosts

Termites penetrate and excavate the roots and continue upwards within the stems. The plant often dies and death may be associated with the presence of soil pathogens, which usually cause rotting, including *Fusarium* spp. and *Phytophthora* spp. (Dominguez-Gil and McPheron, 1992; Piza Júnior, 1992).

Control

The use of tillage operations to reduce populations of termites may change the physical condition of soil and expose the colony to the sun. After tillage, the soil should be treated with Hilban 2.5 ml/liter (Piza Júnior, 1992). The soil must be treated when it is wet to allow the penetration of the insecticidal solution. When the crop is already established, the insecticidal solution must be applied to the soil around the plants in large quantities to reach a depth of 35 cm.

♦ BEES

Benefits

The bee family consists of different species and the carpenter bees are normally counted as beneficial organisms as they enhance the pollination of passion fruit flowers. Passion fruit flowers are cross pollinated species. The floral structure of passion flower does not facilitate self pollination. Bees assisted pollination is usually happening in passion fruit vineyards which help in greater number of fruit setting.

Fig 9: Carpenter Bee

Fig 10: Honey Bee

Hosts

Trigona spinipes damages flowering buds and leaves of various plant species including mulberry, banana, citrus, coconut, mango, rose pine and fig (Silva et al. 1968).

Damage

Honey bee *Apis mellifera* L. (Apidae) is considered a pest since it robs the pollen from the carpenter bees, thereby causing a reduction of fruit set (Akamine *et al*., 1954). Adults of *Trigona spinipes* Fabricius (Apidae) attack leaves, stems, trunk, developing buds, developing fruits, and fruit peduncles of several plant species (Puzzi, 1966; Bastos, 1985; Teixeira *et al*., 1996). *Trigona spinipes* causes malformation of foliage and dropping of flowers, resulting in a reduction in the number of fruits produced per plant. It also attacks developing flowering buds (Fancelli and Mesquita, 1998). The parasitism of larvae of *T. spinipes* by *Pseudohypocera nigrofascipes* Borgn. & Schn (Phoridae) is reported by Silva *et al*. (1968).

Natural Enemies

The most important natural enemy of the larvae of *T. spinipes* is Pseudohypocera nigrofascipes (Silva et al. 1968). They are included in the family Phoridae

Control

To prevent honeybees from robbing passion fruit flowers, more attractive plant species such as eucalyptus and basil can be planted in adjacent areas to passion fruit. Collection of wild swarms is also recommended (Boaretto *et al*., 1994). The control strategies recommended for *T. spinipes* include the destruction of nests near the crop and weekly inspections to verify the occurrence of this pest on flowers. In exceptional cases, chemical control is recommended.

Registered/Suitable pesticides

Nematicide: Carbosulfan 6G, 17 kg/ha, soil application

Insecticides: Chlorpyriphos (Hilban 20EC, 2.5 ml/l)
Imidacloprid (Tatamida 200SL, 0.3 ml/l)
Quinalphos (Ekalux 25EC, 2 ml/l)

Miticide: Dicofol 4 ml/l

Note: Use 500 l/ha for foliar spray and 1 l/m^2 for soil drenching

REFERENCES

Akamine, E.K., Hamilton, R.A., Nishida, T., Sherman, G.D. and Storey, W.B. (1954) Passion Fruit Culture. University of Hawaii, Extension Circular 345, 23 pp.

Back, E.A. and Pemberton, C.E. (1918) The Mediterranean fruit fly in Hawaii. *United States Department of Agricultural Bulletin* 536, 1–118

Barbagallo, S., Cravedi, P., Pasqualini, E. and Patti, I. (1997) *Aphids of the Principal Fruit-Bearing Crops*. Bayer, Milan, 123 pp.

Bastos, J.A.M. (1985) *Principais Pragas das Culturas e Seus Controles*, 3rd edn. Nobel, São Paulo, 329 pp.

Boaretto, M.A.C., Brandão, A.L.S. and São José, A.R. (1994) Pragas do maracujazeiro. In: São José, A.R. (ed.) *Maracujá, Produção e Mercado*. DFZ/UESB; Vitória da Conquista, pp. 99–107.

Brandão, A.L.S., São José, A.R. and Boaretto, M.A.C. (1991) Pragas do maracujazeiro. In: São José, A.R., Ferreira, F.R. and Vaz, R.L. (eds) *A Cultura do Maracujá no Brasil*. FUNEP, Jaboticabal, pp. 136–168

Carter, D. (1992) Butterflies and Moths. Dorling Kindersley, London, 304 pp.

Chacón, P. and Rojas, M. (1981) Biologia y control natural de *Peridroma saucia*, plaga de la flor de la curuba. *Revista Colombiana de Entomologia* 7, 47–53.

Chacón, P. and Rojas, M. (1984) *Entomofauna asociada* a *Passiflora mollissima, P. edulis* f. *flavicarpa* y *P. quadrangularis* en el Departamento del Valle del Cauca. *Turrialba* 34, 297–311.

Chiavegato, L.G. (1963) *Leptoglossus gonagra* – praga do maracujá. *O Agronômico*, 15, 31–36.

Costa, J.M., Correa, J.S., Santos, Z.F.A. F. and Ferraz, M.C.V.D. (1979) *Estudos da Broca do Maracujazeiro na Bahia e Meios de Controle*. EPABA, Salvador, Comunicado Técnico 37, 10 pp.

De Bortoli, S.A. and Busoli, A.C. (1987) Pragas. In: Ruggiero, C. (ed.) *Cultura do Maracujazeiro*. Legis Summa, Ribeirão Preto, pp. 111–123

De Bortoli, S.A. and Busoli, A.C. (1987) Pragas. In: Ruggiero, C. (ed.) *Cultura do Maracujazeiro*. Legis Summa, Ribeirão Preto, pp. 111–123

Dominguez-Gil, O.E. (1998) Fauna fitófaga de parchita maracuyá (*Passiflora edulis* f. *flavicarpa*) en las regiones oriental y suroriental de la cuenca del Lago de Maracaibo, Venezuela: características morfológicas. *Boletín del Centro de Investigaciones Biológicas* 32, 1–66.

Dominguez-Gil, O.E. and McPheron, B.A. (1992) Arthropods associated with passion fruit in western Venezuela. *Florida Entomologist* 75, 607–612.

Echeverri, F., Cardona, G., Torres, F., Pelaez, C., Quiñones, W. and Renteria, E. (1991) Ermanin: an insect deterrent flavonoid from *Passiflora foetida* resin. Phytochemistry 30, 153–155.

Fancelli, M. (1992b) *A Lagarta de Teia do Maracujazeiro*. EMBRAPA/CNPMF, Bahia, Maracujá em Foco 54, 2 pp.

Fancelli, M. (1993) Ocorrência de *Azamora penicillana* (Walk.) (Pyralidae: Chrysauginae) em maracujá silvestre. *Anais da Sociedade Entomológica do Brasil* 22, 121–124.

Fancelli, M. and Mesquita, A.L.M. (1998) Pragas do maracujazeiro. In: Braga Sobrinho, R., Cardoso, J.E. and Freire, F.C.O. (eds) *Pragas de Fruteiras Tropicais de Importância Agroindustrial*. EMBRAPA/SPI, Brasília, pp. 169–180.

Fisher, T.W. (1963) Mass Culture of Cryptolaemus and Leptomastix – Natural Enemies of Citrus Mealybug. University of California Agricultural Experiment Station Bulletin, No. 797.

Flechtmann, C.H.W. (1989) *Ácaros de Importância Agrícola.* Nobel, São Paulo, 189 pp.

Forster, L.D., Luck, R.F. and Grafton-Cardwell, E.E. (1995) *Life Stages of California Red Scale and its*

Parasitoids. University of California, Division of Agricultural Natural Research Publication, 21529

Grasswitz, T.R. and Paine, T.D. (1993) Influence of physiological state and experience on the responsiveness of *Lysiphlebus testaceipes* (cresson) (Hymenoptera: Aphidiidae) to aphid honeydew and host plants. *Journal of Insect Behaviour* 6, 511–528

Haddad, G.O. and Millán, F. (1975) *La Parchita Maracuyá* (Passiflora edulis *f.* flavicarpa *Degener*). Fondo de Desarollo Frutícola, Caracas, Boletim Técnico 2, 82 pp.

Inch AJ (1978) Passion fruit diseases. Queensland Agricultural Journal 104, 479-484.

Mariconi, F.A.M. (1952) Contribuição para o conhecimento do *Diactor bilineatus* (Fabricius, 1803) (Hemiptera: Coreidae), praga do maracujazeiro (*Passiflora* spp.). *Arquivos do Instituto Biológico* 21, 21–42.

Murray, D.A.H. (1976) Insect pests on passion fruit. *Queensland Agricultural Journal* 102, 145–151.

Murray, D.A.H. (1978) Effect of fruit fly sprays on the abundance of the citrus mealybug, *Planococcus citri* (Risso) and its predator,*Cryptolaemus montrouzieri* Mulsant, on passionfruit in southeastern Queensland. *Queensland Journal of Agricultural and Animal Science* 35, 344–346.

Oliveira, C.A.L. (1987) Ácaros. In: Ruggiero, C. (ed.) *Cultura do Maracujazeiro*. Legis Summa, Ribeirão Preto, pp. 104–110.

Oliveira, J.C. and Busoli, A.C. (1983) *Philonis* sp. (Coleoptera, Curculionidae), nova praga do maracujazeiro em Jaboticabal, SP. In: *Resumos do 8 Congresso Brasileiro de Entomologia*. SEB, Brasília, p. 281.

Piza Júnior, C.T. (1992) *Pragas e Doenças do Maracujá*. CATI, Campinas, Comunicado Técnico 96, 9 pp.

Puzzi, D. (1966) Pragas dos Pomares de Citros do Estado de São Paulo. Instituto Biológico de São Paulo, São Paulo, Publicações 116, 58 pp.

Rossetto, C.J., Cavalcante, R.D., Grisi Júnior, C. and Carvalho, A.M. (1974) *Insetos do Maracujazeiro*. Instituto Agronômico, Campinas, Circular Técnica 39, 12 pp.

Rossetto, C.J., Longo, R.S., Rezende, J.A.M. and Branco, E.M.C. (1978) Ocorrência de *Philonis* sp. (Coleoptera: Curculionidae) como praga de maracujazeiro. *Ciência e Cultura* 30, 9.

Santos, Z.F.A.F. and Costa, J.M. (1983) *Pragas da Cultura do Maracujá no Estado da Bahia*. EPABA, Salvador, Circular Técnica 4, 10 pp

Silva, A.G.A., Gonçalves, C.R., Galvão, D.M., Gonçalves, A.J.L., Gomes, J., Silva, M.N. and Simoni, L. (1968) *Quarto Catálogo dos Insetos que Vivem nas Plantas do Brasil, Seus Parasitos e Predadores*, Part II, Vol. 1. Ministério da Agricultura/DDIA, Rio de Janeiro, 622 pp.

Steyskal, G.C. (1980) Two-winged flies of the genus flowers or fruit of species of *Passiflora* (passion fruit, granadilla, curuba, etc.). In: *Proceedings of Entomological Society of Washington* 82, 166–170.

Swaine, G., Ironside, D.A. and Yarrow, W.H.T. (1985) *Insect Pests of Fruit and Vegetables*. Queensland Department of Primary Industries, Brisbane.

Teixeira, C.G. (1994) Maracujá. I – cultura. In: Teixeira, C.G., Castro, J.V., Tocchini, R.P., Nishida, A.L.A.C., Turatii, J.M., Leite, R.S.S., Bliska, F.M.M. and Garcia, E.B. (eds) *Maracujá, Cultura, Matéria-Prima, Processamento e Aspectos Econômicos*, 2nd edn. ITAL, Campinas, pp. 3–131.

Wharton, R.A. (1996) Parasitoids of fruit-infesting Tephritidae – how to attack a concealed host. In: *Abstracts of XX International Congress of Entomology*. Firense, Italia, p. 665.

FRUITS, BENEFITS, PROCESSING, PRESERVATION AND PINEAPPLE RECIPES

Joy P. P. & Minu Abraham, Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala, India, Tel. & Fax: +914852260832, Email: prsvkm@gmail.com

FRUITS

Fruits are nature's wonderful medicines packed with vitamins, minerals, anti-oxidants and many phyto-nutrients without which human body cannot maintain proper health and develop resistance to disease. They also contain pectin, cellulose which stimulates intestinal activities and energy giving substances like oils, fats and proteins. Many fruits have medicinal values. Fruits are a high-moisture, generally acidic food that is relatively easy to process and that offers a variety of flavor, aroma, colour and texture to the diet.

Fruits, eaten raw or consumed as fresh juice are an excellent way to retain and balance moisture level in a body. The low level of sodium in fruits plays an important role for people who avail of salt free diet.

Fruits are an important source of energy. Eating fruit provides health benefits — people who eat more fruits and vegetables as part of an overall healthy diet are likely to have a reduced risk of some chronic diseases. Fruits provide nutrients vital for health and maintenance of our body. However, their availability is seasonal and they are perishable. Hence, they need to be processed to make juices, squashes, jams, etc and preserved.

BENEFITS

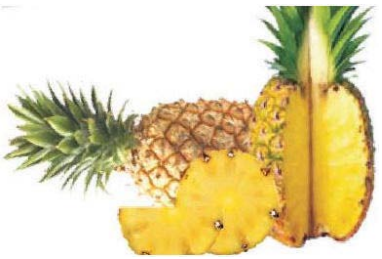
Health Benefits

- Eating a diet rich in fruits as part of an overall healthy diet may reduce the risk of heart disease, including heart attack, obesity, type 2 diabetes and stroke and may protect against certain types of cancers.
- Eating fruits rich in potassium may lower blood pressure and may also reduce the risk of developing kidney stones and help to decrease bone loss.
- Eating fruits that are lower in calories may be useful in helping to lower calorie intake.
- Fruits hydrate the body because they are made up of 90 - 95 percent water. Water is an important nutrient. It is responsible for transporting nutrients around the body, regulating body temperature, keeping joints moist and getting rid of waste products in the body.
- Fruits keep the body regular because they are rich in fiber, which is essential for the smooth movement of food in the body's digestive system. Fruits help maintain easy bowel action and eating fruits every day will prevent constipation.
- Fruits give the body energy because they contain carbohydrates, which are the body's main source of energy. Carbohydrates in fruits are mainly sugar, which break down easily and make a quick source of energy.

Nutrients

- Most fruits are naturally low in fat, sodium, and calories. None have cholesterol.
- Fruits are sources of many essential nutrients that are under consumed, including potassium, dietary fiber, vitamin C and folate (folic acid).
- Diets rich in potassium may help to maintain healthy blood pressure. Fruit sources of potassium include bananas, prunes and prune juice, dried peaches and apricots, cantaloupe, honeydew melon and oranges.
- Dietary fiber from fruits, as part of an overall healthy diet, helps reduce blood cholesterol levels and may lower risk of heart disease. Fiber is important for proper bowel function. It helps reduce constipation. Fiber-containing fruits help to provide a feeling of fullness with fewer calories. Whole or cut-up fruits are sources of dietary fiber; fruit juices contain little or no fiber.
- Vitamin C is important for growth and repair of all body tissues, helps heal cuts and wounds and keeps teeth and gums healthy.

Pineapple: Nature's Healing Fruit



- Pineapple is one of the popular fruits and is liked by majority of the people irrespective of their age group. Pineapple is an important food which can be eaten fresh or eaten in a processed form. It is composed of nutrients which are good for human health.
- Pineapples are nutritionally packed members of the bromeliad family. This delightful tropical fruit is high in the enzyme Bromelain and the antioxidant vitamin C, both of which play a major role in the body's healing process. Bromelain is a natural anti-inflammatory molecule that has many health benefits and encourages healing. Pineapple fruit is very low in Saturated Fat, Cholesterol and Sodium. It is a good source of Dietary Fiber.
- Pineapples are packed of vitamin C and fiber – important for the immune and digestive systems.
- Pineapples have anti-inflammatory effects which are good for those long hard days and those heroic sporting injuries.
- They contain the enzyme Bromelain which is thought to aid digestion
- It regulates the gland and found to be helpful in cases of goiter

Pineapples are beneficial for the treatment of the following

- Dyspepsia (chronic digestive disturbance)
- Bronchitis (inflammation of the bronchial tubes)
- Catarrh (secretions from mucous membranes)
- High Blood pressure
- Arthritis (diseases of the joints)
- Intestinal worms
- Nausea (includes morning sickness and motion sickness).

FRUIT PROCESSING

Fruits are highly perishable items which needs processing to make it durable. Fruit processing is any deliberate change in a fruit that occurs before it's available for us to eat. Processing methods extend the shelf life of fruits.

Fruit processing has three major aims:

1. To make fruit safe (microbiologically & chemically).
2. To provide good quality products with good flavor, color, texture and taste.
3. To make convenient fruits products

Fruits should be prepared for preservation as soon as possible after harvesting within 4 to 48 hours. As time passes spoilage increases rapidly. Fruit processing involves many steps.

Cleaning and washing

First, the fruits should be cleaned thoroughly to remove any adhering dirt or pesticide residues. This cleaning process usually involves washing the product with running water.

Sorting

To achieve a uniformly sized product, fruits and vegetables are sorted immediately after cleaning according to their size, shape, weight or colour. Sorting by size is especially important if the products are to be dried or heated, because their size will determine how much time will be needed for these processes.

Peeling

Many types of fruits have to be peeled in order to be preserved. This can easily be done with a stainless steel knife. It is extremely important that the knife be made of stainless steel because this will prevent the discoloration of the plant tissues.

Cutting

Cutting is important in order to get uniform pieces for heating, drying and packing. Fruits are usually cut into cubes, thin slices, rings or shreds. The cutting utensils have to be sharp and clean to prevent micro-organisms from entering the food.

Blanching

Blanching is a slight heat treatment, using hot water or steam that is applied mostly to fruits before canning or freezing. It is done by immersing fruits in water at a temperature of 90-95°C. The result is that fruits become soft and the enzymes are inactivated. Blanching is done before a product is dried in order to prevent unwanted colour and odour changes and an excessive loss of vitamins.

FRUIT PRESERVATION

Fruit preservation is the process of treating and handling food to stop or slow down fruit spoilage, loss of quality, edibility or nutritional value and thus allow for longer fruit storage.

Preservation usually involves preventing the growth of bacteria, fungi (such as yeasts), and other micro-organisms as well as retarding the oxidation of fats which causes rancidity. Fruit preservation can also include processes which inhibit visual deterioration, such as the enzymatic browning reaction in apples after they are cut, which can occur after fruit cutting.

Many processes designed to preserve food will involve a number of fruit preservation methods. Preserving fruit by turning it into jam, for example, involves boiling (to reduce the fruit's moisture content and to kill bacteria, yeasts, etc.), sugaring (to prevent their re-growth) and sealing within an airtight jar (to prevent recontamination).

Maintaining or creating nutritional value, texture and flavor is an important aspect of fruit preservation.

Preservation methods

Drying

Drying is one of the most ancient fruit preservation techniques, which reduces water activity sufficiently low to prevent bacterial growth. Drying is the partial removal of water from solid foods. It is one of the oldest methods of food preservation. It was traditionally carried out in the presence of sun.

Refrigeration

Refrigeration preserves fruit by slowing down the growth and reproduction of micro-organisms and the action of enzymes. Refrigerators should be set to below 4°C to control the growth of micro-organisms. This lowered temperature also reduces the respiration rate of fruits and retard the spoilage.

Commercial and domestic refrigerators improved the shelf life of foods such as fresh fruits and salads to be stored safely for longer periods, particularly during warm weather.

Vacuum packing

Vacuum-packing stores food in a vacuum environment, usually in an air-tight bag or bottle. The vacuum environment strips bacteria of oxygen needed for survival, slowing spoiling. Vacuum-packing is commonly used for storing dried fruits to reduce loss of flavor during oxidation.

Freezing

Freezing is also one of the most commonly used processes commercially and domestically for preserving fruit including prepared fruit stuffs which would not have required freezing in their unprepared state. Lowering the temperature below the freezing point of the product stops microorganisms from growing and reduces the activity of enzymes. Fruits are heat treated (blanched) before freezing to eliminate enzymes. Home freezers are held at -10°C,

commercial freezers are under -18°C. At this temperature, the growth of micro-organisms is almost stopped.

Pasteurization

Pasteurization is a process of heating a product at a specific temperature for a controlled period of time to destroy the most heat resistant vegetative pathogenic organism. The process is also applied for fruit juices and juice products.

Canning

Canning involves cooking food, sealing it in sterile cans or jars and boiling the containers to kill bacteria.

Importance of Sugar & Preservatives in Fruit Preservation

Sugar is used to preserve fruits, either in syrup with fruit such as apples, pears, peaches, apricots, plums or in crystallized form where the preserved material is cooked in sugar to the point of crystallization and the resultant product is then stored dry. This method is used for the skins of citrus fruit (candied peel) and ginger.

Preservative / food additives can be antimicrobial; which inhibit the growth of bacteria or fungi, including mold or antioxidant; such as oxygen absorbers, which inhibit the oxidation of fruit constituents. Common antimicrobial preservatives include calcium propionate, sodium nitrate, sodium nitrite; sulfites (sulfur dioxide, sodium bisulfate, potassium metabisulfite, etc) and antioxidants which include BHA (Butylated Hydroxy Anisole) and BHT (Butylated Hydroxy Toluene).

Pickling in Fruits

Pickling is a method of preserving fruit in an edible anti-microbial liquid. Pickling can be broadly categorized into two categories: chemical pickling and fermentation pickling.

In chemical pickling, the fruit is placed in an edible liquid that inhibits or kills bacteria and other microorganisms. Typical pickling agents include brine (high in salt), vinegar, alcohol, and vegetable oil, especially olive oil but also many other oils. Many chemical pickling processes also involve heating or boiling so that the food being preserved becomes saturated with the pickling agent. Common chemically pickled fruits include mango and lemon.

In fermentation pickling, the food itself produces the preservation agent, typically by a process that produces lactic acid.

STORAGE

Always store the preserved food in a cool place, at a temperature below 20°C. Keep glass bottles and jars out of light. The storage area has to be dry and with a consistent temperature. Moisture will make tins rust.

Materials Used in Fruit Processing and Preservation

Fig 1: Steel bowl

Fig 2: Teaspoon (tsp) & Table spoon (tbsp)

Fig 3: Nonstick pan

Fig 4: Cup

Standard Measurements

1/4 tsp	1 ml
1/2 tsp	2 ml
1 tsp	5 ml
1 tbsp	15ml (3 tsp)
1/4 cup	50 ml
1/3 cup	75 ml
1/2 cup	125 ml
2/3 cup	150 ml
3/4 cup	175 ml
1 cup	250 ml (225 g)

PINEAPPLE RECIPES

1. JUICE

Pineapple juice tastes best when chilled and it is an ideal fruit drink to consume during the hot summer days. Fresh pineapple juice contains about 75% of vitamin C. It acts as a natural antioxidant. It promotes cell growth and tissue repair. Pineapple juice also contains vitamin B6, which helps our body to regulate blood sugar and also promote a healthy immune system.

Ingredients (For 750 ml of juice) = 725 g

500 gram	Pineapple
250 gram	Sugar
250 ml	Water
1/2 cup	Crushed ice

Fig 5: Ingredients for Juice

METHOD

- Peel the skin and cut into small pieces.
- Blend the pineapple pieces, sugar and required amount of water in a blender.
- Then filter it to get the clear juice.
- Transfer into glass and add some crushed ice.
- Serve chilled.

Fig 6: Juice

2. PINEAPPLE JUICE CONCENTRATE

Pineapple juice concentrate is prepared from fresh, ripened pineapples to provide the essential flavour and nutrition, in a convenient, ready to use ingredient form for processed beverage and food applications.

The juice concentrate is derived when the fruit juice is evaporated and water is removed, yielding a thicker liquid product, which is a concentrate of the original fruit juice. The product having less water is easier to handle, easier to store, and because of its higher solids content, becomes easier to stabilize. These products do much better under frozen and even refrigerated storage conditions.

METHOD

- Peel the skin and cut pineapple into small pieces.
- Blend the pineapple pieces in a blender.
- Then filter it to get the clear juice.
- Cook the pineapple juice with sugar and citric acid.
- Boil it well by stirring continuously.
- When the sugar dissolves completely, add dissolved sodium benzoate.
- Take off from fire and allow to cool.
- Pour into sterilized bottles and seal.

Fig 7: Pineapple juice concentrate

3. SQUASH

Pineapple squash should be prepared from fully matured and ripe pineapple fruits free from insect infestation, diseases etc. For preparing this juicy and delicious pineapple squash, firstly clean the pineapple and peel the skin thickly. Grate the pineapple and filter the grated pineapple through a clean cloth and collect the juice out of it and keep it aside. Squash is a concentrated form of fruit drink. The pineapple squash is generally diluted 2-3 times with water at the time of consumption and chilled with ice cubes and served. Preparing the Pineapple squash is very simple and easy.

Ingredients (For 500 ml of squash = 475 g)

1 cup	Pure fresh pineapple juice
2 cups	Sugar
1 cup	Water
1 tsp	Citric acid
1/8 tsp	Yellow food colour
1/8 tsp	KMS (Potassium metabisulphite)

Fig 8: Ingredients for Squash

METHOD

- Bring sugar and water to boil in a deep vessel.
- Simmer to make sticky syrup, which is not one thread.
- Add dissolved citric acid, take off from fire.
- Cool and add juice, dissolve KMS.
- Stir till well blended.
- Pour into sterilized bottles and seal.
- Refrigerate opened bottle.

Serving: Add 1 tbsp of squash in 150 ml water and serve.

Fig 9: Squash

4. JAM

Pineapple jam is made from mature pineapple fruit which is boiled with sugar and other ingredients. For preparing pineapple jam the selection of fruit is very important. Pineapple must be perfectly ripe. The young fruit contains acids and could affect jam quality. It is unsuitable, if it contains large amount of water and unattractive color.

Pineapple jam is a nutritious spread on various foods. The pineapple jam can be eaten as a spread on toast and as a filling for bread, buns, biscuits, cakes, and other pastries. It can be used to make ice creams, yogurts, milk shakes and cocktails.

Ingredients (For 350 g of Jam = 375 ml)

250 gram	Pineapple
250 gram	Sugar
1/2 tsp	Citric acid
1/2 tsp	Pectin powder
1/4 tsp	Lemon yellow color
1/2 tsp	Pineapple essence
2 1/2 cups	Water

Fig 10: Ingredients for Jam

METHOD

- Cook the pineapple pulp with water on a low fire.
- Stir it continuously with a wooden ladle.
- While it boils slowly add sugar into it. Boil it well by stirring continuously.
- Add pectin powder and stir continuously.
- When the jam is done, add citric acid, lemon yellow colour and pineapple essence to it
- Remove from fire and pour into a bottle. When the jam cools, close the mouth of the bottle

Fig 11: Jam

To test whether the jam is formed, pour some jam on a dry plate. Allow it to cool and tilt the plate. If the jam is ready, it will fall in flakes.

5. KESARI

Semolina (rawa) kesari is simple South-Indian dessert mainly prepared during festive and special occasions. Adding fruits make it tastier. Fresh pineapple chunks are being used for this recipe. Pineapple kesari is a delightful delicious South Indian sweet Recipe.

Ingredients (For 500 g of Kesari = 525 ml)

1 cup	Rava
1/2 cup	Ghee
500 gram	Fresh Pineapple
2 cups	Water
11/4 cup	Sugar
few	Cardamoms powdered
2 tbsp	Cashew nuts and raisins (fried in 2 tsp ghee)
A pinch	Salt
A few drops	Pineapple essence

Fig 12: Ingredients for Kesari

METHOD

- ❖ Cut, slice pineapple and grind partially; powder cardamom.
- ❖ Heat a pan (no ghee), put the rava into the pan and heat it until golden brown with constant stirring. Put the rava into a dry plate.
- ❖ Put 1 tea spoon of ghee from 1/2 cup given, fry cashew nuts, raisins and keep.
- ❖ In the same frying pan, add rava, fry for 2 seconds; add 2 cups of water, mix well and bring to boil; boil in low flame, till rava is half cooked; add ground pineapple pieces, mix well and cook for few seconds.

Fig 13: Kesari

- ❖ Add sugar, cardamom powder and mix well; add ghee, stir well, cook till the mix is thick and leaves the sides of the pan.
- ❖ Transfer kesari onto a big bowl and Garnish with fried cashew nuts and raisins; serve hot or cold.

6. PICKLE

Pickles are generally spicy; they can also be made sweet by adding sugar. Spicy pickles are very important item in Indian meal. Fruits can also be used for making pickles. Pickling may also increase the shelf life of food. Fruits, such as papaya and pineapple are also sometimes pickled.

Ingredients (For 500 g pickle = 525 ml)

250 gram	Pineapple
3 tbsp	Coconut oil
1 tsp	Ground mustard seeds
1/2 tsp	Mild chilli powder
1/4 tsp	Turmeric

Fig 14: Ingredients for Pickle

50 gram	Green chilly
25 gram	Small onion
50 gram	Garlic
A few	Springs fresh curry leaves
1/4 tsp	Black pepper, finely ground
100 gram	Sugar
100 ml	Vinegar

Fig 15: Pickle

METHOD

- Cut the pineapple into eight long wedges, and then remove the tough core from each wedge. Chop each pineapple wedge into small pieces, about the size of a dice.
- Heat the coconut oil in a saucepan, add the spices and fresh curry leaves; when they fizzle add the pineapple.
- Add the sugar and vinegar and cook gently until the mixture is thick and slightly jammy.
- Transfer pickle into a bowl.

7. HALWA

Pineapple halwa is a pineapple flavored mouth watering sweet dish. It is a delicious dessert dish which can be served as a snack or after meal. It is very tasty and easy to prepare.

Ingredients (For 400 g of Halwa = 425 ml)

1-1/2 cup	Pineapples (grated)
150 gram	Sugar
1/2 cup	Khoa (grated)
1/2 cup	Milk
1/2 tsp	Cardamom Powder
1/2 glass	Water
2 tbsp	Ghee
2 or 3	Almonds

Fig 16: Ingredients for Halwa

METHOD

- Take water in a pan and heat it on a medium flame. Now add the grated pineapple in it for boiling.
- Then add sugar and ghee. Stir continuously. Then add milk and Khoa and mix gently till the water evaporates.
- Cook it for at least 10 minutes at low flame
- Now remove from the flame and sprinkle cardamom powder.
- Finally garnish with almonds and serve hot.

Fig 17: Halwa

8. CANDY

Candy is a very sweet food. Sugar syrup and fruits are its basic ingredients. Pineapple candy is one of the delicious fruit products and increases the shelf life of candy by drying process.

Fig 18: Ingredients for Candy

Ingredients (For 500 g of candy): 525 ml

500 gram	Pineapple (moderate size)
250 ml	Water
4 cups	Sugar

METHOD

Fig 19: Candy

- Peel the pineapple; remove eyes, core and wash
- Slice into cubes.
- Prepare the syrup, 2 parts sugar to 1 part water.
- Boil the pineapple in the syrup for 20 minutes.
- Soak in syrup overnight.
- Strain and wash well in water.
- Dry in solar drier for 16-20 hours.
- Let cool.
- Roll over sugar and wrap in cellophane.
- Put in plastic bags; seal open end of bag with the flame of a candle.

9. PUDDING

Pineapple pudding is a healthy dessert, as it is made up of pineapple fruit. The fresh ingredients make the dessert even more delicious. For pudding, the pineapple used should be fresh or canned. The best two ingredients of the pineapple pudding dessert are the crushed pineapple and the fresh cream. Pineapple Pudding is a very tasty and easy recipe.

Ingredients (For 1 kg of pudding = 1.025 liter)

250 gram	Pineapple
10	Slices soft white bread
100 gram	Soft butter
350 ml	Milk
1 tbsp	Lime juice
1/4 tsp	Ground nutmeg

Fig 20: Ingredients for pudding

1/4	tsp	Ground cinnamon
1/4	tsp	Ground clove
2	large	Egg white (beaten)
2	large	Egg yolk (beaten)
250	gram	Granulated sugar
1	tsp	Vanilla essence
1	tsp	Cardamom powder
50	gram	Raisins

Fig 21: Pudding

METHOD

- Preheat oven to 350°F (175°C).
- Cook the pineapple with half cup of water and 2 tbsp of sugar and drain it. Keep the pineapple aside.
- Heat a pan, put the milk into the pan and allow to boil with constant stirring.
- When the milk is boiling add the bread powder and cook it for 10 minutes. Keep aside for cooling.
- In a medium mixing bowl, combine butter, sugar and egg yolk. Mix well. Add ground cinnamon, nutmeg powder, ground cloves, cardamom powder and vanilla essence. Add cooked pineapple to it. Beat until well mixed.
- Pour over cooled milk and bread mixture. Fold the beaten egg white little by little to this mixture.
- Place it in a pudding dish and sprinkle with raisins and cashew nuts.
- Bake in the preheated oven for 45 minutes, until the surface is golden brown.

10. PAYASAM

Payasam / Kheer is an Indian sweet dessert. Pineapple payasam is made with pineapple, chowery (Sago) and milk. Nuts such a pistachio, cashew and almonds along with raisins, saffron and cardamom are roasted in ghee and added to give a rich feel, taste and good appearance.

Ingredients (For 1 liter of payasam = 975 g)

250	gram	Pineapple
3/4	cup	Grated jaggery
50	gram	Chowari
2	tbsp	Ghee

Fig 22: Ingredients for Payasam

1/2	cup	Water
1	cup	Coconut milk (first milk)
2	cups	Coconut milk (second milk)
2	tsp	Cardamom powder
15	gram	Cashew nut
10	gram	Raisins

Fig 23: Payasam

METHOD

- Roast the nuts and raisins in 1 tablespoon of ghee and keep it aside.
- Boil the pineapple pieces in a thick bottom pan, along with a little water.
- When the pineapple is done, add the ghee and fry it well.
- Add grated jaggery and cook till the color changes to dark brown.
- When it is nicely done, add the third extract of the coconut milk and cook till the payasam is thick and add cardamom powder.
- Lower the flame and add the second extract followed by the first extract.
- When the first extract begins to boil, add the washed chowari.
- When the chowari is cooked and payasam is nicely done, remove from flame.
- Add the roasted cashew nuts and raisins.
- Remove from flame and allow cooling.

11. PULISSERY

Pulissery is a traditional Kerala dish made using yogurt (curd) and grated coconut**.** Sour curd is used for making pulissery and vegetables or fruits are often added to pulissery to balance the sourness.

Ingredients (For 1 liter of Pulissery = 975 g)

2	cups	Pineapple cut into pieces
2		Green chili
2 or 3		Curry leaves
1/2	tsp	Turmeric powder
1	cup	Yogurt / Curds
Salt		to taste

Grind to Paste

1	cup	Grated coconut (fresh)
1/2	tsp	Jeera / cumin seeds
2	pods	Garlic
2 or 3		Curry leaves
2		Green chilli

Fig. 24: Ingredients for Pulissery

For Seasoning

1 tsp	Mustard
4	dry red chilli
1/4 tsp	Fenugreek seeds
A few	Curry leaves
1 tbsp	Coconut oil

Fig 25: Pulissery

METHOD

- Clean and cut the pineapple into small pieces.
- Cook the pineapple pieces along with a little water, turmeric powder, chilli powder, & salt until it turns to soft and tender.
- Grind and make a paste of coconut, jeera, 2 green chilli, 2 - 3 curry leaves with little water
- Add to the cooked pineapple. Also add whipped yogurt, mix well and bring to a boil. Cook for a minute. Take off from stove.
- For seasoning - heat oil in a pan, add mustard. When it pops, add fenugreek seeds, whole red chilli and curry leaves. Add to the pulissery.
- Serve as a side dish with rice.

12. PINEAPPLE UPSIDE DOWN CAKE

Cakes can be made using flour and fruits as filling. Butter and sugar enhance its taste, sweetness and appearance. Cake also contains protein nutrients from eggs that are used as a binder for all ingredients. Cake is an excellent source of fats and oils through its shortening and frosting. Fruits like pineapple, carrots and apples, can be incorporated as filling or the body of the cake.

Ingredients (For 1 kg cake = 1.025 liter)

1 cup	Maida
A pinch	Salt
1 tsp	Baking powder
1tbsp	Vanilla essence
1/4 cup	White sugar
1/4 cup	Butter
1 large	Egg
1/4 cup	Low fat milk

Fig. 26: Ingredients for upside down cake

For topping:

1 1/2 tbsp	Butter
1/4 Cup	Cup brown sugar
4-5	Pineapple slices (tinned and drained)
6-7	Glazed cherries

Fig 27: Upside down cake

METHOD

- Preheat oven to 175^{o}C. Grease and flour a round baking pan. Prepare the topping by melting butter in a pan and add brown sugar. As the sugar melts and foams, cook on medium flame for a minute and pour into the baking pan. Over this sugar layer, place pineapple slices and in the center of each pineapple piece place a glazed cherry. Keep aside.
- Sieve Maida, baking powder and salt in a bowl.
- In another bowl, cream butter and sugar. Use a hand blender to make a smooth creamy mixture. Add the beaten egg and combine well. Add vanilla essence and combine.
- Fold the Maida mixture little by little alternating with milk. Do not over beat; just fold them dry till there is no trace of any flour.
- Pour batter over the fruit layer. Bake in pre heated oven for 45 minutes or till a tooth pick inserted into the cake comes out clean. Place on a wire rack to cool, slice and serve at room temperature.

13. PINEAPPLE BALL

Pineapple ball is a simple snack dish made with semolina or rava. It is a popular sweet which is prepared from ghee, sugar, rava, cardamom and dry fruits. It can be served any time of the day.

Ingredients (For 500 g of ball = 525 ml)

1	cup	Rava
1/2	cup	Ghee
500	gram	Fresh Pineapple
1	tsp	Seasame
11/4	cup	Sugar
1/2	tsp	Cardamoms (powdered)
2	tbsp	Cashew nuts and raisins (fried in 2 tsp ghee)

Fig: 28: Ingredients for pineapple ball

METHOD

Fig 29: Pineapple ball

- Cut the pineapple into small pieces and cook it with low fire.
- Grind the pineapple to make a paste.
- Add ghee to the heating pan. Put the rava into the pan and heat it until golden brown with constant stirring. Put the rava into a dry plate.
- Put 1 tea spoon of ghee from 1/2 cup given, fry cashew nuts, raisins and keep. Fry seasame in low fire.
- Prepare the syrup, 2 parts sugar to 1 part water.
- Boil the pineapple in the syrup for 5 minutes and add rava to it.
- When it reaches in the form of making ball add cashew nuts, raisins, powdered cardamom and seasame. Make balls of convenient size and serve into a bowl.

14. PINEAPPLE ICE-CREAM

Ice cream is a frozen dessert usually made from dairy products, such as milk and cream, and often combined with fruits or other ingredients and flavours. Most varieties contain sugar, although some are made with other sweeteners. Pineapple ice cream is a sweet summer treat that is easy to make at home.

Fig: 30: Ingredients for Ice-cream

Ingredients (For 1 liter of Ice-cream = 975 g)

1 litre	Milk
1/4 cup	Custard powder
1 tin	Condensed milk
1/2 cup	Pineapple
1/4 cup	Sugar syrup
2 drops	Pineapple essence

Fig 31: Ice-cream

METHOD

- Boil the milk, custard powder and condensed milk to make the custard.
- Heat the pineapple with sugar syrup till the pineapple gets well cooked.
- Mix the custard, cook pineapple and add the pineapple essence to it.
- Beat the mixture well using an egg beater.
- Convert it to an ice-cream tray and freeze it for 4 – 6 hours.
- Serve it chilled.

14. PINEAPPLE LIME

Fig 32: Ingredients for pineapple lime

Ingredients (For 750 ml pineapple lime = 725 g)

500 g	Pineapple
250 g	Sugar
50 ml	Water
250 g	Lime

METHOD

Fig 33: Pineapple lime

- Peel the skin of pineapple and cut into small pieces.
- Cut the lime into small pieces.
- Blend the pineapple pieces, lime, sugar and required amount of water in a blender.
- Then filter it to get the clear juice.
- Transfer into glass and add some crushed ice.
- Serve chilled.

15. TROPICAL PINEAPPLE COLADA COCKTAIL

Fig34: Tropical Pineapple Colada Cocktail

Ingredients For Tropical Pineapple Colada Cocktail

2	cups	Pineapple juice
1	cup	Pineapple
1/2	cup	Rum
1/2	cup	Coconut cream
2	tbsp	Palm sugar
1	cup	Crushed ice

METHOD

- Put all ingredients in a blender and blend until smooth.
- Transfer to a serving jug and serve immediately over crushed ice.

16. PINEAPPLE VODKA

Fig 35: Pineapple Vodka

Ingredients for Vodka

250 gram	Fresh pineapple
250 gram	Vodka

METHOD

- Pour vodka over the pineapple until all the fruit is covered.
- Place a lid and then store in the fridge for ten days.
- Peel and cut your fresh pineapple into chunks, then place these in a glass container that has a lid.

17. WINE

Wine is an alcoholic beverage made from fermented grapes or other fruits. Wines made from fruits besides grapes are usually named after the fruit from which they are produced (for example, pomegranate wine, apple wine and pineapple wine) and are commonly called fruit wine.

Pineapple wine is made from the juice of pineapples. Fermentation of the pineapple juice takes place in temperature-controlled vats and is stopped at near-dryness. The result is a soft, dry, fruit wine with a strong pineapple flavour.

Fig 36: Wine

Ingredients For Wine

1 kg	Pineapple
2 kg	Sugar
10 cup	Water
2 tbsp	Yeast

METHOD

- Wash the pineapples and cut into small pieces. Don't remove its skin.
- Boil it for about 5 minutes with water and 1 kg sugar.
- When cool, add yeast and store in an air tight mud vessel for 20 days.
- Stir the content daily with a wooden ladle.
- After 21 days, filter the wine through a fine cloth. Do not squeeze the contents. Add the remaining sugar and store it for another 21 days without stirring.

18. VINEGAR

Processing pineapple into vinegar is a good way of turning over ripe, blemished or surplus fruits, discarded cores, peels and trimmings into money.

Although not as popular as coconut vinegar, pineapple vinegar is already being exported in small quantities.

Pineapple vinegar can be produced by alcohol and acetic acid fermentation.

Alcohol fermentation

- Wash the pulp of the ripe fruits.
- Mix well and one part mashed fruits with three parts of water.
- Press the mixture through a cheese cloth with double thickness.
- Add 1.5 kg of sugar for every 9 liters of the diluted juice, and pasteurize it at 65^{o}C for 20 minutes.
- Cool and transfer the mixture in a suitable container.
- Add two tablespoon of yeast.
- Cover the container with the clean cheese cloth or loose cotton wad.
- Allow the solution to ferment from four to seven days until no more carbon dioxide bubbles form.
- Strain the liquid through the clean cheese cloth to remove the yeast and other solid materials.
- Pasteurize the alcoholic liquid at 65^{o}C and allow it to cool.

Acetic acid fermentation

- To the alcoholic solution, add 2 liters of the mother vinegar or starter for every volume of the formulation indicated above.
- Mother vinegar may be obtained from the National Institute of Science and Technology (NIST), Orissa or elsewhere.
- Set it aside undisturbed for one month or until maximum sourness (acidity) is obtained.
- To develop desirable aroma and flavour, allow the vinegar to age in the barrels, or earthen jars filled to capacity.
- Filter the vinegar and pasteurize it to kill microorganisms before bottling the product.
- If clear vinegar is desired, add the well-beaten white of two eggs for every 10 liters of vinegar and stir it until the egg white coagulates.
- The clear vinegar is obtained by filtering.

CONCLUSION

Pineapple is a tropical fruit which is consumed fresh or in a processed form. It contains nutrients which are good for human health. It also contains antioxidants and protease. It is useful against malignant cell formation, thrombus formation and inflammation.

Processed pineapples are consumed worldwide and processing industries are trying out or using new technologies to retain the nutritional quality of the pineapple fruit. This is to meet the demand of consumers who want healthy, nutritious and natural products. Pineapple wastes from these processing industries can be utilized to produce methane, animal feed and manure.

PROTOCOL FOR MICROPROPAGATION OF PINEAPPLE (MD-2)

Joy P. P., Anjana R. & Prince Jose, Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686670, Muvattupuzha, Ernakulam, Kerala, India, Tel. & Fax: +914852260832, Email: prsvkm@gmail.com

The objective of micropropagation of pineapple is to produce large number of disease free planting material and to satisfy the large need of pineapple planting materials.

Stages of micropropagation of Pineapple

Selection of mother plant, Preparation of explants, Fresh inoculation, Multiplication, Shooting, Rooting, Planting out, Primary hardening, Secondary hardening, Field planting.

1. Selection of mother plant

The micropropagation work can be facilitated by the strict selection of planting material at the onset of the production cycle. Suckers can be collected from field, gene banks, and farmer's field or from isolated nursery area. High yielding good quality disease free plants are selected as Mother Plants.

2. Preparation of explants and fresh inoculation

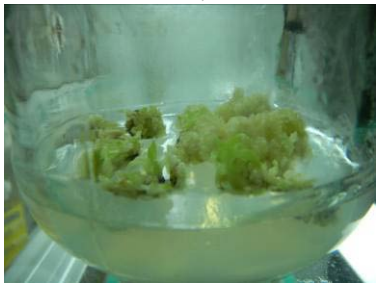
- Roots and leaf sheaths are removed from the sucker, and basal portion of the sucker is cut and trimmed to a size of 12x12x15 mm
- Keep the explants under running tap water for 30', then soaked in cleansol (detergent) for 30 minutes and are shaken continuously
- Wash with distilled water to remove the detergent particles
- Treat with fungicide [SAAF (0.05%)+INDOFIL(0.1%)+ BAVISTIN(0.1%)] for 30' followed by distilled water wash
- They are then transferred to laminar air flow chamber for further sterilization process
- Inside the laminar flow chamber, the explants are Stirred with 70% ethanol for 2'
- Wash with sterile water
- After that the explants are Stirred with 0.1% $HgCl_2$ for 5'
- Three rinsing of 5' each with sterile water
- The explants are trimmed to a final size of 1 x1x1cm, [sometimes the explant may dipped in Gentamycin(2ml/L)] in sterile conditions inoculated to PA1 (MS+3mg/l BA) media,
- Incubate at 25+/- 2 ^{o} C for 21 days

Diagrammatic Representation of Micropropagation of Pineapple

Selection of Explant (0 day)

Explant Preparation (0 day)

Fresh inoculation (0 day)



Multiplication (4 Months)

Field Planting (21 Months)

Hardening (10 Months)

Rooting (9 Months)

Shooting (6 months)

Diagrammatic Representation of fresh inoculation of Pineapple

Pineapple Sucker (explant)

Explant size minimized

Running tap water wash for 30'

Explant in soap solution for 30'

Treated with Bavistin(0.1%),Indofil(0.1%) and SAAF(0.05%) for 30'

Washed out using distilled water

Transferred to sterile bottles

Sterile water wash

0.1% $HgCl_2$ treatment for 5' with continuous shaking

Sterile water wash for 5' continuous shaking, thrice

Cut the explant size to 1 cm

MD2 explant inoculated to MS+3mg/l BA media

MULTIPLICATION

The inoculated explants will show bulging and new bud formation within 7 days. After 21 days they are transferred to 5BA (MS+5mg/l BA) media for more bud formation. Again after 21 days the cultures are transferred to multiplication media.

3. Multiplication

This step consists of separating buds, culturing them up to form callus. If they have grown bigger than optimum size, transferring them to fresh culture medium PA2 (MS+4mg/l BA+1mg/l NAA) and again going through the same cycle of activities for another subculture. This step is repeated for eight to ten cycles.

4. Shooting

Multiplied callus in PA2 media are transferred to PA3 (MS+3mg/l BA+0.5mg/l NAA) media for the development of shoots. It takes two months of duration for the proper development of shoots in the media.

5. Rooting

After two months of time developed shoots are placed in to the IN (HMS+1mg/l IBA+1mg/l NAA) media for the generation of roots. The development of root in pineapple is very slowly, and it takes normally about 3 months.

6. Planting out and Hardening

Fully rooted plants in vitro are selected for planting out. Plants are first grown in mist chamber for acclimatizing with climate outside the lab **(Primary Hardening)**. After 2-3 weeks time, they are moved to green house to get adjusted with field conditions **(secondary Hardening).** Plants are planted in potting mixture. After 12 months the plants are ready for field planting.

For the planting of a mature hardened plant to the field, we require 20-22 months of duration from the date of inoculation, and we get more than thousand plants from an explant that is inoculated in the artificial PA1 media.

Tentative production estimates (Nos)

Responding Explants	Periods (Months) (If recovery ≥ 90%)						
	12	15	18	21	24	27	30
1	400	900	1300	1600	2000	2200	2400
5	2000	4200	6200	8000	10000	11000	12000
10	4000	8200	12200	16000	20000	22000	24000
50	20000	40200	60200	80000	100000	110000	120000
100	40000	80200	120200	160000	200000	220000	240000

One Biotechnologist & 6 racks for every 25000 TC plants (2500 bottles of 10 plants)
5750 TC plants (575 bottles of 10 plants) for every rack

PROTOCOL FOR MICROPROPAGATION OF BANANA

Joy P. P., Anjana R. & Prince Jose, Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686670, Muvattupuzha, Ernakulam, Kerala, India, Tel. & Fax: +914852260832, Email: prsvkm@gmail.com

Micro propagation of Banana

Plant tissue culture is a collection of experimental methods of growing large number of isolated cells or tissues under sterile and controlled conditions.

Banana is one of the world's most important fruit crop. It is grown in all type of tropical agriculture systems. The yield depends not only on the quality of soil and fertilization, but largely upon the control of the diseases.

An important objective of micro propagation of banana is to produce large number of disease free planting material and to satisfy the large need of banana planting materials.

Stages of micro propagation of banana

Selection of mother plant

⇩

Preparation of explants

⇩

Fresh inoculation

⇩

Multiplication

⇩

Rooting

⇩

Planting out

⇩

Primary hardening

⇩

Secondary hardening

⇩

Field planting

Diagrammatic Representation of Micropropagation of Banana

Selection of mother plant (0 day)

Banana Inflorescence

Preparation of explants

Fresh inoculation (0 day)

Multiplication (90 days)

Rooting (6 months)

Planting out & primary hardening (7 months)

Secondary hardening (8 months)

Field planting (9 months)

Joy P. P. 2013. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala
Tel.: 0485-2260832, 9446010905; Email: prsvkm@kau.in, prsvkm@gmail.com; Web: www.kau.edu/prsvkm, http://prsvkm.tripod.com

Selection of mother plant.

The micro propagation work can be facilitated by the strict selection of planting material at the onset of the production cycle. Suckers and inflorescences can be collected from field, gene banks, and farmer's field or from isolated nursery area. Criteria for the selection mother plant- they are disease free, high yield and good quality plants.

Preparation of explants and fresh inoculation

1. **Banana shoot tip (sucker)**

- Wash the suckers thoroughly in tap water, roots and leaf sheaths are removed, and basal portion of the corm is cut and trimmed to a size of12*12*15 mm.
- Keep the explants under running tap water for 30 minutes, then soaked in cleansole (detergent) for 30 minutes and are shaked continuously.
- Wash with distilled water to remove the detergent particles.
- Treat with fungicide (SAAF+INDOFIL) for 30 min followed by distilled water wash.
- They are then transferred to laminar air flow chamber for further sterilization process.
- Inside the laminar flow chamber, the explants are treated with 70% ethanol for 2 min,
- Wash with sterile water.
- After that the explants are treated with 0.1% Hgcl2 for 5 min
- Three rinsing of 5 minutes each with sterile water.
- The explants are trimmed to a final size of 8*8*10 mm, in sterile conditions inoculated on BA1 (MS+3mg/l BA1) media,
- Incubate at 25+/- 2 o C dark for 21 days.
- The media used for inoculation is changed after 21 days for 3 times, unless the phenolics released into the medium may inhibit the growth.

2. **Banana inflorescence**

- The bracts with the male flowers are removed until they become too small (3cm in length).
- Wash the inflorescences thoroughly in running tap water for 30 min
- Soaked in cleansole detergent to remove surface contaminants for 30 min in shaker.
- Wash with distilled water for about 5 times to remove soap solution.
- Fungicide treatment (0.05%Saaf+0.1 % indofil) for 30 min in a shaker.
- Wash with distilled water for 5 times to remove fungicide..
- Transfer to sterile bottles inside the laminar air flow chamber.
- Rinse with sterile water once.
- 70% ethanol wash for 2 minutes.
- Wash with sterile water once.
- Treat with 0.1%Hgcl2 for 5 minutes.
- Rinsing of 5 minutes wash with sterile water for 3 times.

OUTSIDE LAF

Banana sucker (explant)

Explant size minimized

Running tap water wash for 30'

Explants in soap solution for 30'

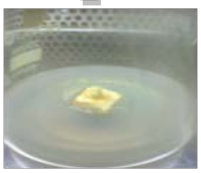
Treated with Bavistin(0.1%)+Indofil(0.2%)+SAAF(0.1%) for 30'

Washed out using distilled water

Further sterilization in LAF

INSIDE LAF

MULTIPLICATION



Inoculated to media

Cut the explants size to 8x8x10mm

Sterile water wash for 5'continuous shaking, thrice

0.1% $HgCl_2$ treatment for 5' with continuous shaking

Sterile water

70% alcohol wash for 2'

Transferred to sterile bottles

Fresh Inoculation of Banana shoot tip

Joy P. P. 2013. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala
Tel.: 0485-2260832, 9446010905; Email: prsvkm@kau.in, prsvkm@gmail.com; Web: www.kau.edu/prsvkm, http://prsvkm.tripod.com

OUTSIDE LAF

Banana inflorescence (Explant)

Explant size minimised

Running tap water wash for 30'

Explant in soap solution for 30'

Washed out using distilled water

Further sterilization in LAF

INSIDE LAF

MULTIPLICATION

Explant inoculated to media

Size minimized

Sterile water wash for 5' continuous shaking thrice

0.1% $HgCl_2$ treatment for 5' with continuous shaking

Sterile water wash

70% alcohols wash for 2'

Fresh inoculation of banana inflorescence

The inoculated explants will show bulging within 7 days, and may release phenolic compounds. Aftrer 21 days they are transferred to fresh media (BA1). About three media change will be done. After the third media change it will be subcultured to BA2 media for multiplication and formation of buds.

Multiplication

Multiplication step is for rapid production of clones. This step consists of separating shoots, culturing them up if they have grown bigger than optimum size, transferring shoot or sections of the shoot to fresh culture medium and again going through the same cycle of activities for another subculture. This step is repeated for seven to eight cycles.

Rooting

After the transfer of callus sections to BA2 media, the plants with 2-3 cm length are transferred to HB(half basal) media for the generation of roots.

Planting out and Hardening

Fully rooted plants in vitro are selected for planting out. Plants are first grown in mist chamber for acclimatizing with climate outside the lab. After 2-3 weeks time, they are moved to green house to get adjusted with field conditions. Plants are planted in potting mixture, after 5-6 days they are transferred to soil within a plastic cover. After 2-3 months the plants are ready for field planting.

For the planting of a mature hardened plant to the field, we require 9 months duration from the date of inoculation, and we get more than thousand plants from an explant that is inoculated in the artificial BA media.

Tentative production estimates (Nos)

Responding Explants	Period (Months) (If recovery ≥ 90%)			
	9	12	15	18
1	25	500	800	1200
5	125	2500	4000	6000
10	250	5000	8000	12000
50	1250	25000	40000	60000
100	2500	50000	80000	120000
500	12500	250000	400000	600000
1000	25000	500000	800000	1200000

One Biotechnologist & 6 racks for every 25000 TC plants (2500 bottles of 10 plants)
5750 TC plants (575 bottles of 10 plants) for every rack

BASIC FRUIT ANALYSIS OF PINEAPPLE: A LABORATORY MANUAL

Joy P. P. and Soumya K.K. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686670, Muvattupuzha, Ernakulam, Kerala. Tel: 0485-2260832, Email: prsvkm@gmail.com; Web: www.kau.edu/prsvkm

COLLECTION OF PINEAPPLE FRUIT SAMPLES

- **Selection of fruits**: All the fruits are required for the physical or chemical studies. Selection is randomized or individual fruits are selected for our studies. Select a pineapple that is plump and fresh-looking. The leaves in the crown should be fresh and green, and the body of the pineapple firm. The first method is to look at the scales on the side of the pineapple. These are called eyes. If a pineapple has eyes of a uniform size all the way to the top, that's a good sign the pineapple is ripe. Avoid the ones where the eyes near the top are significantly smaller than the ones at the base. The second is to smell the bottom of the pineapple where the cut stem is located. It should have a faint pineapple scent, but should not smell too strong or fermented. Too strong a pineapple smell means that the pineapple is overripe and might be mushy. Picking the small leaves on the top of the pineapple near the center of the rosette can also tell you if it is ready, as long as the leaves are not wilted.

- **Method of plucking**: Plucking of pineapple fruit is actually done with a sharp cutting instrument, can be a cutter or a knife which gives a clean and smooth cut on the stock. Try to get a uniform stock length. Proper documentation and labeling were done prior and after harvesting.

- **Sorting**: Fruits for analysis are sorted. The fruits showing any symptom of infection or damage on surface should be scrupulously rejected and only healthy are sorted for conducting study.
- **Surface Cleaning**: After cutting off the stalks or other foliage parts which remain attached to the fruits after harvest, they should be meticulously cleaned before use. Surface cleaning can be done by using clean dry/ moistened cloth piece.
- **Bringing to analytical laboratory**: Fruits of pineapple are brought to the laboratory as soon as possible after they are plucked and stored in the same atmosphere conditions to maintain the minimal change of the physio-chemical conditions.

DETERMINATION OF CONSITITUENTS BY PHYSICAL METHODS

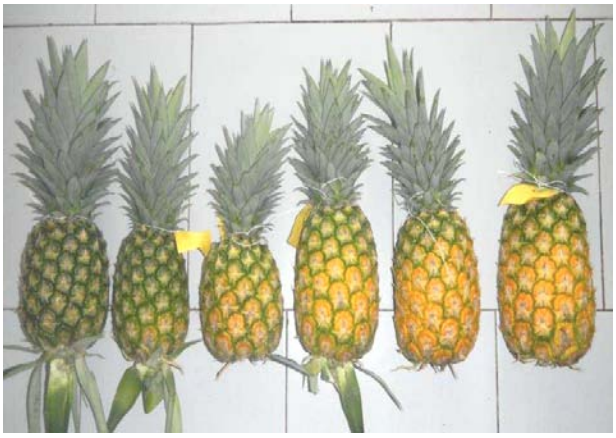
There may in fact, be a large number of physical characteristics of fruits that are worthy to test. Those are as follows.

- **Weight:** Weight of a fruit is considered to be an important factor in judging its compactness, maturity, juice content, carbohydrate and other chemical constituents. It is done by physical balance. Balance should be properly set, placed and leveled, accuracy ensured before use, weighing done accurately and the reading noted carefully.
- **Volume:** Volume that is the size of a fruit is another important factor. In market consumers prefer large-sized ones for many fruits. Volume of the fruit can be determined by measuring the volume of a liquid that is water which is actually displaced by it.

- **Overall Length:** The length of a fruit is referred to by many as the space, that is, straight line distance between its stalk-end and the stylar end. It appears to be more appropriate to consider the total length of a fruit, which may be termed as its overall length. This can be done by slide calipers, L-shaped sets etc.
- **Maximum width:** To refer width, the diameter of a fruit in its centre is emphasized. It can be done by measuring the distance from the extreme points at two sides using slide calipers, L-shaped sets etc.
- **Shape:** Fruits belonged to a species or a variety of it has some characteristic shape of their own, although variation within some limit is not considered to be an uncommon feature.

Firmness/ pulp firmness: Firmness of a healthy fruit is linked to the degree of its physiological maturity. With progress of development, maturation and ripening either in the pre-harvest or in the post-harvest condition, the fruits undergo gradual softening to a greater or a lesser extent depending on species, varieties, environment and the use of agro-inputs. Enzymatic conversion of pectic compounds may cause this. This can be measured by penetrometer, by hand-feeling, and by pin drop method.

- **Peel colour:** The colour of the fruit surface is an important factor in




determining a appeal to the consumers. The change in colour is due to accumulation of one or more forms of pigments in different combinations. This can be measured by, the use of colour-dictionary, eye-estimation etc.

- **Peel smoothness:** The peels of fruits get smoothened with advancement of their development, maturation and ripening. In pineapple, the eyes become less raised. It can be judged by hand feeling of a group of testers.
- **Peel wax**: Plant wax is an ester of a higher fatty acid with long chain alcohol other that glycerol. It can be done by simply rubbing the surface with fingers or using a tissue paper.
- **Peel thickness:** The thickness of the peel is considered to be a character of importance of many fruits. To measure the peel thickness, it has to be excoriated from the fruit. It should be carefully cut out with a knife in order to separate from the inner part of the fruit. Slide – calipers is used to measure the thickness.

- **Colour of the edible part**: The colour of the inside part undergoes change with progress of their development. The color changes are due to various combinations of chlorophylls, carotene, xanthophylls, anthocyanin, anthoxanthin etc. So, by estimating the relative intensity of the colour may be done by chemical analysis of pigments or by several group eye estimations. Juice colour can be determined by optical density value, which is done in colorimeter. Absorbance is obtained against distilled water.

- **Edible matter content:** Although in fruit culture, the quantitative productivity of a fruit crop is conventionally determined in considering the yield of the whole fruits or the number of them that are harvested from a given number of trees or an unit area of land, it is more apposite to determine how much matter that is consumable to the human beings has actually been obtained from the same trees or plants or the same area of land. In pineapple outer rachis, bract, perianth and pericarp all fused together. In it usually we get approximately 68% of human consumable matter. It can be measured by

Weight of consumable matter (g)/weight of whole intact fruits (g) x 100

Or

Volume of juice (ml)/ weight of the whole intact fruit (g) x 100

- **Flavour:** Flavour is charm to a fruit. Flavour is due to the existence of adiverse type of volatile compounds eg., alcohols, esters, aldehydes, ketones, ethers, halides, hydrocarbons and others in different proportions. It will change with development. It can be measured by ultra-sophisticated chemical procedures or by smelling power of expert persons.
- **Seed content:** Presence of seeds in a fruit is considered a demerit or a merit. Consumers always prefer seedless fruits. But seeds are required for the production of seedlings. Seed content can be measured by conventional way, cutting and removed by rubbing and washing. Records to keep usually include the number of bold and less bold seeds per fruit and weight of one hundred seeds. Viability of seeds also sometimes considered.

DETERMINATION OF CHEMICAL CONSTITUENTS

❖ Ascorbic acid or Vitamin C

Vitamin C or ascorbic acid is an enediol isomer of 2-keto-L-gluconolactone with a configuration similar to that of L-glucose. Oxidation of ascorbic acid gives rise to dehydro-ascorbic acid and both forms are physiologically active.

✓ Principle:

Titrimetric estimation of vitamin C is conventionally done using 2, 6-dichlorophenol indophenol dye solution. This dye is blue in alkaline solution and red in acidic solution. Ascorbic acid reduces the dye to a colorless form. Reaction is quantitative and specific for ascorbic acid at pH 1.0-3.5.

✓ Reagents:

(a) 4 % Oxalic acid : 40 gm of oxalic acid is dissolved in 1000ml of distilled water (W/V)

(b) DCPIP Dye solution: Dissolve 0.250 g of sodium salt of 2,6-dicholorophenol indophenols in about 500ml of water containing 0.210 g of $NaHCO_3$ and dilute to 1 liter of water. Store the solution in refrigerator and standardize it with freshly prepared standard solution of ascorbic acid every time just before use # (Sadasivam and Manikam)

(c) Standard ascorbic acid ($C_6H_8O_6$): 0.01 % ascorbic acid is dissolved in oxalic acid

✓ Procedure

1. Take 5 gm of fruit sample (filtered juice) make up to 100 ml with 4% oxalic acid.
2. Take 5 ml sample from the 100 ml and add 10 ml 4% oxalic acid and titrate against the dye 2, 6-dicholorophenol indophenol.
3. The end-point is determined by the appearance of pink colour which should persist for at least 15 seconds.
4. Standardization of the dye solution: The dye solution is needed to be standardized simultaneously. For this 5ml of standard ascorbic acid solution is taken in conical flask and to this, 10ml of 4% oxalic acid is added. Mixed well and titrated against DCPIP.

✓ **Results**

Titer values are noted and this will be in a range between 0.1 -1 ml.

✓ **Calculation**

60.6* T.V= ----- mg/100g

0.5mg/V1ml (3.3) *V2 (T.V)/5ml*100ml/Wt of sample (5gm) *100

❖ **Total Titratable acidity**

Acids are important constituents in fruits as together with sugars, they determine quality and taste of the fruits. Maturity of many fruits for their harvest is also judged from their level of acids along with sugars, or the soluble solids. Fruits contain organic acids and among inorganic acids, only phosphoric acid is present. Acids that are accumulated in fruits are largely synthesized in leaves and are translocated to fruits.

In some fruits, one or more acids may be present in relatively high amount than the other acids and accordingly, these are referred to as predominant acids respective to these fruits. For example, citric acid is the predominant acid in fruits like citruses and also in strawberry, currants etc., while malic acid is predominant in apple, cherry, plum etc., tartaric acid and malic acids in grapes, and bromelain in pineapple.

✓ **Principle:**

The total acidity of a fruit could be determined by titrating a known amount of aqueous extract of it against an alkali solution of known normality. It is expressed as equivalence of any organic acid, eg. Citric, malic etc.

✓ **Reagents**

(a) Sodium hydroxide solution: Make 50 ml 0.1N NaOH by dissolving 0.2 gm of NaOH in 50 Ml water

(b) Phenophthalein ($C_{20}H_{14}O_4$) indicator: Approx. 0.5 % in 80 % ethanol

✓ **Procedure**

1. Take 25 ml sample add 100 ml water and heat for 10 minutes.
2. Make up the sample to 250 ml with water.
3. Take 10 ml from the sample.
4. Few drops of phenolphthalein solution is added and shaken well.
5. A burette is filled with the 0.1 Normal sodium hydroxide solution after washing and rinsing.
6. Titration is done and the end-point is determined by the appearance of pink colour and its persistence for at least few seconds.

✓ **Results**

Titer value ranges from 0.1-1.5 ml

✓ **Calculation**

1ml of 0.1 N NaOH solution can neutralize 0.064g of citric acid. Therefore, percentage of total titratable acidity in the sample as equivalence of citric acid=

V1 (T.V) *N (0.1)*0.064*250 (50)/50*100/w (5)

0.128 * T.V= ----- %

❖ REDUCING SUGAR

In fruits, both reducing and non-reducing sugars are present in varying amount.

Reducing sugars are those hexose ($C_6H_{12}O_6$) sugars, which can reduce compounds such as alkaline (ammoniacal) silver nitrate solution, cupric salt solution etc., because they themselves are oxidized.

Hexose sugars are divided into 2 main groups, which are aldo-hexose and keto-hexose. Aldo-hexose or aldose contains aldehyde group and keto-hexose or ketose contains ketone group. Aldehyde are strong reducing agents. Hexose sugars which contain aldhyde groups *eg*., glucose, galactose, mannose etc., are reducing sugars. Ketones are however, more resistant to oxidation than aldehydes, because it involves the breaking of a relatively stable C-C bond. Hence, they do not ordinarily reduce alkaline silver nitrate or cupric salt solution. But those fructose contains ketone, it is able to reduce readily as easily oxidizable CO-CH_2OH group is present in it and it acts as reducing sugar.

Non-reucing sugar *eg*., sucrose is a disaccharide and cannot reduce alkaline silver nitrate or cupric acid solution.

✓ Principle:

When sugars are extracted and titrated, the reducing sugars only take part in the reaction in making reduction, but the non-reducing sugars that are present in it, do not take part in reduction and remains as such. Accordingly, only the reducing sugars are estimated by titration.

✓ Reagents:

(a) Fehlings Solution – 5ml Fehlings A + 5ml Fehlings B + 20 ml Water
(b) 45 % Lead acetate ($C_2H_3O_{22}Pb$, $3H_2O$): 45g of Lead Acetate in 100 ml water
(c) 22 % Oxalic Acid: 22g of Oxalic Acid in 100 ml water

✓ Procedure:

1. Take 25 g of sample (filtered juice) and heat for 3 minutes, till it turns to a curd like appearance.
2. Add 2ml of 45% Lead Acetate and wait for 2 minutes.
3. Add 22% Oxalic acid to the sample to remove the excess Lead acetate.
4. Wait till a yellowish tint appears and add NaOH until the bubble retains in the sample to neutralize the solution.
5. Make up to 250 ml and titrate against hot Fehling's solution. Add Methylene Blue at the end point and heat.
6. End point of the reaction is a green colour appearance. On addition of methylene blue and heating red colour appears.

✓ Results

Titer value range is 8-11 ml

✓ Calculation

0.05*250/V (T.V) *100/w (25)

50/T.V = g of glucose/100g of juice

❖ TOTAL SUGAR

✓ Principle

The non-reducing sugars which are not titratable are first hydrolyzed to reducing sugars. Thus after hydrolysis, the non-reducing sugars are converted to reducing sugars while the reducing sugars that are already present in the sample remain unchanged. Accordingly, all the sugars that are present after hydrolysis remain as reducing sugars. This is conveniently termed as total sugars.

✓ Reagents:

(a) Fehlings Solution – 5ml Fehlings - A + 5ml Fehlings - B + 20 ml Water
(b) 45 % Lead acetate ($C_2H_3O_{22}Pb$, $3H_2O$): 45g of Lead Acetate in 100 ml water
(c) 22 % Oxalic Acid: 22g of Oxalic Acid in 100 ml water
(d) NaOH drops to neutralize

✓ Procedure

1. Procedure is same for reducing sugar the volume made up to 250 ml.
2. Out of the 250ml sample solution, take 50 ml and add 5 gm citric acid.
3. Heat the sample and make up to 250 ml with water.
4. Titrate against the Fehling's solution.
5. End point of the reaction is a green colour appearance.
   On addition of methylene blue and heating brick red colour appears.

✓ Results

Titer value range is 7-9 ml

250/T.V= g of glucose/100g of juice

✓ Calculation

0.05*250/T.V *250/50*100/W (25)

❖ NON REDUCING SUGAR

The non-reducing sugars present in the sample may be determined from the values of the total and the reducing sugars as follows.

Percentage of non-reducing sugars = [Percentage of (Total sugars)- (Reducing sugars)] x 0.95

Non Red = (Total – Reducing) *0.95

TOTAL SOLUBLE SOLIDS

Total soluble solids (TSS) of a given sample of fruit juice represent the various chemical substances present in it in soluble form. It indicates a measure of sugars present in the sample. The amount of TSS present in the juice of a fruit is also considered to be a reliable index in judging its maturity. In accordance with, the harvest-maturity of many fruits is assessed in considering the TSS of their juices.

✓ Principle

The TSS of a given fruit juice sample is determined in a quicker way with the help of a Refractometer, which is also known as hand or pocket Refractometer. The instrument works on the principle of refractive index of the sample and gives the refractive index as ^{o}Brix.

✓ Requirements

A hand- Refractometer. A dropper or a glass-rod. Blotting paper. Absorbent cotton. Rectified spirit. Distilled water

✓ Procedure

1. The lid, that is, covering plate of the Refractometer which rests over the prism-plate and is attached with it at the base end with a hinge is unfolded backward. By doing so, both the lid and the prism-plate are exposed.

2. The lid and the prism-plate are then carefully and scrupulously washed with jet of clean water to ensure that they have no stain on their surfaces.

3. Water adhered on the prism-plate and the lid as well as the surrounding parts of them, if any is completely wiped off with blotting paper or absorbent cotton.

4. The lid of the prism-plate is then washed with distilled water and the water adhered on them is blotted out. The cleaning is best done by rubbing the lid and the prism-plate gently and carefully with absorbent cotton, soaked with rectified spirit.

5. Then, with the help of a previously cleaned dropper or a glass-rod, a drop of distilled water is carefully dropped on the surface of the prism-plate. The lid is folded forward and placed over the prism-plate to cover it. At this position, the lid and the prism-plate are firmly held together with fingers to avoid unfolding of the lid.

6. The Refractometer is held to point towards light. The eyepiece of it is brought close to any eye of the observer who should look into the eye-piece to have a view of the image of the scale. The scale-focusing knob should be conveniently rotated to adjust it at such a position where the scale is most clearly visible. The shaded part would be seen to intersect the unshaded part at zero position of the scale which indicates no reading with respect to distilled water. If its not there then the reading should brought to zero by rotating the scale-calibrating screw.

7. The lid is then folded backward. The distilled water that remains adhered over the lid and the prism-plate is completely blotted out and these are dried in air for a few minutes.

8. A clear sample of fruit juice, TSS of which is to be determined is taken in the dropper, or a drop may be taken with the glass-rod. A drop of juice is, then carefully placed on the prism plate.

9. Reading of the juice sample as ^{o}Brix is obtained and amount of TSS is expressed accordingly.

✓ Correction factor

It should be noted that temperature is an important factor in the measurement of refractive index. The calibrating drop of distilled water should in fact, be at the same temperature as with the temperature of the juice sample. The determination should be done at 20^{o} C.

RECENT TRENDS IN BIOLOGY

Soumya K.K. and Joy P. P. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686670, Muvattupuzha, Ernakulam, Kerala. Tel: 0485-2260832, Email: prsvkm@gmail.com; Web: www.kau.edu/prsvkm

INTRODUCTION

Biotechnology has emerged as an area of activity having tremendous impact on virtually all domains of human welfare and holds a lot of promises and surprises in some of the techniques for the present and future. It is a collection of technologies that capitalize on the attributes of biological molecules. It will help to improve the ability to customize therapies based on individual genomics; prevent, diagnose, and treat all types of diseases rather than rely on rescue therapy and provide breakthroughs in agricultural production and food safety. The development has accelerated and today the trends are characterized in the 'omics' – metabolomics, transcriptomics, proteomics, etc. The efforts in metabolic engineering have started to yield organisms with new 'synthetic' metabolic pathways for production of chemicals as well as materials. New developments in microelectronics, microfluidics, and data management have had an enormous impact on biotechnology.

Biotechnology was initially very much focused on medical applications, but now there is more focus on biosociety, that is, biotechnology to replace petrochemistry. Results are seen, for example, in the biofuel market, which is expanding rapidly. This development has a strong influence on the research topics studied today. A trend that started in the early 1980s was process integration that is to combine two or more unit operations into one. This would reduce the work load and also reduce the loss of product that routinely happens in each processing step. Membrane technology has been used to recycle the biocatalyst (often microbial cells) to the bioreactor while the product stream is processed for harvesting the product molecule. Then, the stream may either be sent back to the bioreactor or discarded. Very often, a process was used for production of one compound and all of what remained from the fermentation broth was regarded as waste.

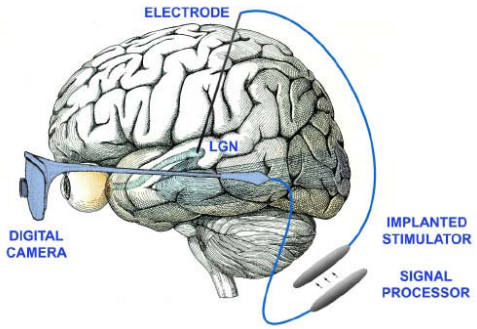
When no more valuable products can be produced, then residue from the biorefinery is used for biogas production. Municipal waste-water treatment, and now it has also expanded into industrial wastewater treatment as well as the destruction of toxic chemicals in dedicated reactors. The introduction of affinity tags, for example, the histidine tags, has made isolation and purification far easier than before. The tags make it possible to selectively catch the target protein from a complex medium. Also, the tools for separation and purification have undergone remarkable improvements. Membrane technology was promising, but often caused problems due to material limitations and lack of operation range with regard to the size of the compounds to be isolated. Gel matrices underwent a similar development, from being soft and pressure-sensitive to becoming robust and suitable to process particulate-containing material. Another example illustrating biotech development is in the biosensor area. To monitor low concentrations of antigens using immunobiosensors was very difficult, both because of lack of access to good and reproducible preparations of antibodies and lack of stability in the electronics on which the biosensor was based. It is a privilege to have been involved in biotech research during this period

of rapid development, both at the basic level and in supporting technologies. Here we tried to furnish few studies which are going on recently.

COUNTDOWN: BIONIC HUMANS: TOP 10 TECHNOLOGIES

Scientists are getting closer to creating a bionic human, or at least a $6 million one. Today, we can replicate or restore more organs and various sundry body parts than ever before. From giving sight to the blind to creating a tongue more accurate than any human taste bud, gentlemen, we have the technology.

Bionic Eyes



When you're blind, being able to see even the basics of light, movement and shape can make a big difference. Both the Argus II Retinal Prosthesis, currently in FDA trials, and a system being developed by Harvard Research Fellow Dr. John Pezaris record basic visual information via camera, process it into electronic signals and send it wirelessly to implanted electrodes. The Argus II uses electrodes implanted in the eye, which could help people who've lost some of their retinal function. Dr. Pezaris' system, still in the early stages of research, would bypass the eyes entirely, sending visual data straight to the brain. Both systems will work best with people who could once see because their brains will already know how to process the information. "The visual brain depends on visual experience to develop normally," Pezaris explained.

Regrown Bone

Since the 1960s, researchers have known about proteins that can prompt bone tissue to grow its own patches for missing or damaged parts. Unfortunately, that technology never worked perfectly, often growing the wrong type of tissue or growing bone where bone shouldn't be. In 2005, researchers at UCLA solved the problem, using a specially designed protein capable only of triggering growth in specific types of cells. Called UCB-1, the protein is now used to grow new bone that can fuse and immobilize sections of vertebrae, relieving severe back pain in some patients.

Portable pancreas

An artificial pancreas, capable of monitoring a person's blood sugar and adjusting the level of insulin to meet their body's needs, will likely be on the market within a few short years, said Aaron Kowalski, director of strategic research projects at the Juvenile Diabetes

Research Foundation. Kowalski said the device would initially be a combination of two existing technologies: an insulin pump and continuous glucose monitor. The contraption could help insulin-dependent diabetics lead more normal lives and make it easier for them to avoid the disfiguring and life-threatening side effects of having too little or too much blood sugar.

Inhuman taste

The tongue can be a powerful tool, but also a highly subjective one, said Dean Neikirk, professor of computer and electrical engineering at the University of Texas at Austin. When food companies want to create the same flavor every time, they turn to the electronic tongue, a device developed by Neikirk and his team to analyze liquids and pick out their exact chemical make-up. Neikirk's tongue uses microspheres, tiny sensors that change color when exposed to a specific targets, such as certain kinds of sugars. The result is a system that can't replace the person who says, "This tastes good!" but can make sure the chemistry of good taste is reliably replicated.

New limbs

Amputees can now use a prosthetic arm the same way they'd use a real one: By the power of thought. Developed by Dr. Todd Kuiken of the Rehabilitation Institute of Chicago, the "bionic arm" is connected to the brain by healthy motor nerves that used to run into the patient's missing limb. These nerves are re-routed to another area of the body, such as the chest, where the nerve impulses they carry can be picked up by electrodes in the bionic arm. When the patient decides to move her hand, the nerves that would have sent the signal to real hand send it to the prosthetic one instead. Now, Dr. Kuiken's team is working on improving the arm, using surviving sensory nerves to communicate the feeling of temperature, vibration and pressure from the bionic arm to the patient's brain.

Smart knee

The knee isn't a part of the body you'd expect to think for itself, but the RHEO, a prosthetic knee developed by MIT artificial intelligence researchers Hugh Herr and Ari Wilkenfeld, really does have a mind of its own. Earlier electronic knee systems usually had to be programmed by a technician when the patient first put them on. The RHEO knee, on the other hand, creates realistic, comfortable motion on its own, by learning the way the user walks and by using sensors to figure out what kind of terrain they're walking on. The system makes walking with a prosthetic leg easier and less exhausting.

Wearable kidney

For people with failing kidneys, basic necessities of life like removing toxins from the blood and keeping fluid levels balanced requires hours hooked up to a dialysis machine the size of a clothes dryer. But a new, portable artificial kidney, small and light enough to fit on a belt system, could change that. Despite its small size, the automated, wearable artificial kidney (AWAK), designed by Martin Roberts and David B.N. Lee of UCLA, actually works better than traditional dialysis because it can be used 24 hours a day, seven days a week, just like a real kidney.

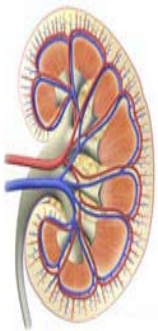
Artificial cells

Sometimes, when you need to deliver drugs to just the right spot in the body, a pill or an injection won't cut the mustard. Daniel Hammer, professor of bioengineering at the University of Pennsylvania, has a better method: artificial cells, made from polymers, which can mimic the ease with which white blood cells travel through the body. Called c, these fake cells could deliver drugs directly where they're needed, making it easier and safer to fight off certain diseases, including cancer.

Prosthetics for your brain

Replacing a part of your brain isn't as simple as replacing a limb, but in the future it could be. Theodore Berger, a professor at the University of Southern California, created a computer chip that could take the place of the hippocampus, a part of the brain which controls short-term memory and spatial understanding. Frequently damaged by things like Alzheimer's and strokes, a hippocampus implant could help maintain normal function in people who'd otherwise be severely disabled. Berger is still testing this implant, but he'd like to see more. He even wrote a book, "Toward Replacement Parts for the Brain," in 2005.

FROM TEENS' SLEEPING BRAINS, THE SOUND OF GROWING MATURITY



By monitoring the brain waves of sleeping teenagers and children, scientists confirm that the brain prunes away neuronal connections during the transition to adulthood.The research, published in the February 15 issue of American Journal of Physiology: Regulatory, Integrative, and Comparative Physiology, also confirms that electroencephalogram, or EEG, is a powerful tool for tracking brain changes during different phases of life, and that it could potentially be used to help diagnose age-related mental illnesses. It is the final component in a three-part series of studies carried out over 10 years and involving more than 3,500 all-night EEG recordings. The data provide an overall picture of the brain’s electrical behavior during the first two decades of life.

NEW MRI 'FINGERPRINTING' COULD SPOT DISEASES IN SECONDS

Each body tissue and disease has a unique fingerprint that can be used to diagnose problems before they become untreatable. By using new magnetic resonance imaging (MRI) technologies to scan simultaneously for various physical properties, researchers say it may be possible to differentiate white matter from gray matter from cerebrospinal fluid in the brain in about 12 seconds—and potentially even faster in the near future. The technology has the potential to make an MRI scan standard procedure in annual check-ups. A full-body scan lasting just minutes would provide far more information and ease interpretation of the data, making diagnostics far less expensive compared to today's scans. As reported in Nature, a magnetic resonance imager uses a magnetic field and pulses of radio waves to create images of the body's tissues and structures. Magnetic resonance fingerprinting, MRF for short, can obtain much more information with each measurement than a traditional MRI. Griswold likens the difference in technologies to a pair of choirs.

CAN PLANTS ACTUALLY TALK AND HEAR?

Though often too low or too high for human ears to detect, insects and animals signal each other with vibrations. Even trees and plants fizz with the sound of tiny air bubbles bursting in their plumbing. And there is evidence that insects and plants "hear" each other's sounds. Bees buzz at just the right frequency to release pollen from tomatoes and other flowering plants. And bark beetles may pick up the air bubble pops inside a plant, a hint that trees are experiencing drought stress. Sound is so fundamental to life that some scientists now think there's a kernel of truth to folklore that holds humans can commune with plants. And plants may use sound to communicate with one another. If even bacteria can signal one another with vibrations, why not plants, said Monica Gagliano, a plant physiologist at the University of Western Australia in Crawley.

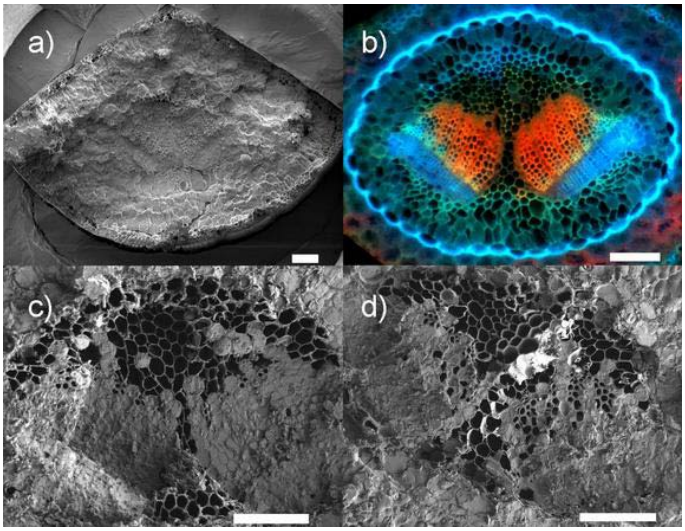


Figure: A Ponderosa Pine needle scanning electron microscope image. What we see is that the xylem (in red) embolizes as the leaves get more dehydrated. a) fully hydrated at minus 112 degrees Fahrenheit (minus 80 degrees Celsius (cryoSEM); b) fully hydrated, but imaged at room temperature with epifluorescence microscopy; c) cryoSEM of a dehydrated needle; and d) cryoSEM of a severely dehydrated needle. Panels b,c, and d are zoomed in compared to panel a.

OBESITY AND DIABETES IDENTIFIED WITH A GENETIC 'MASTER SWITCH'

A gene linked to type 2 diabetes and cholesterol levels has been identified as a 'master regulator' by a team at King's College London and the University of Oxford. The researchers found that the gene controls the behaviour of other genes found within fat tissues in the body. As fat plays a key role in susceptibility to metabolic diseases such as obesity, heart disease and diabetes, the regulatory gene could be a possible target for future treatments to fight these diseases.

The KLF14 gene was already known to be linked to type 2 diabetes and cholesterol levels but, until now, its role or function was unknown. The researchers examined over 20,000 genes in subcutaneous fat biopsies from 800 UK female twin volunteers. They found an association between the KLF14gene and the expression levels of multiple distant genes found in fat tissue, suggesting it acts as a master switch to control these genes. This finding was then confirmed in an independent sample of 600 subcutaneous fat biopsies from Icelandic subjects. The genes found to be controlled by KLF14are linked to a range of metabolic traits, including obesity, cholesterol, insulin and glucose levels, highlighting the interconnectedness of metabolic traits.

IMMORTAL LINE OF CLONED MICE CREATED

Japanese researchers have created a potentially endless line of mice cloned from other cloned mice. They used the same technique that created Dolly the sheep to produce 581 mice from an original donor mouse through 25 rounds of cloning, the scientists report in the March 7 issue of the journal Cell Stem Cell. "This technique could be very useful for the large-scale production of superior-quality animals, for farming or conservation purposes," study leader Teruhiko Wakayama of the RIKEN Center for Developmental Biology in Kobe, Japan, said in a statement.The researchers used a cloning technique called somatic cell nuclear transfer, in which a cell nucleus containing one individual's genetic information is inserted into an egg cell whose nucleus has been removed. Dolly the Sheep became the first cloned mammal in 1996 using this technique. Many other animals have

been cloned since, but the technique has had a low success rate and attempts to "reclone" animals have often failed.

SCIENTISTS REPROGRAM ALPHA CELLS TO FIGHT DIABETES

For years researchers have been searching for a way to treat diabetics by reactivating their insulin-producing beta cells, with limited success. The "reprogramming" of related alpha cells into beta cells may one day offer a novel and complementary approach for treating type 2 diabetes. Treating human and mouse cells with compounds that modify cell nuclear material called chromatin induced the expression of beta cell genes in alpha cells, according to a new study that appears online in the Journal of Clinical Investigation. Both type 1 and type 2 diabetes are caused by insufficient numbers of insulin-producing beta cells. In theory, transplantation of healthy beta cells - for type 1 diabetics in combination with immune suppression to control autoimmunity - should halt the disease, yet researchers have not yet been able to generate these cells in the lab at high efficiency, whether from embryonic stem cells or by reprogramming mature cell types. Alpha cells are another type of endocrine cell in the pancreas. They are responsible for synthesizing and secreting the peptide hormone glucagon, which elevates glucose levels in the blood.

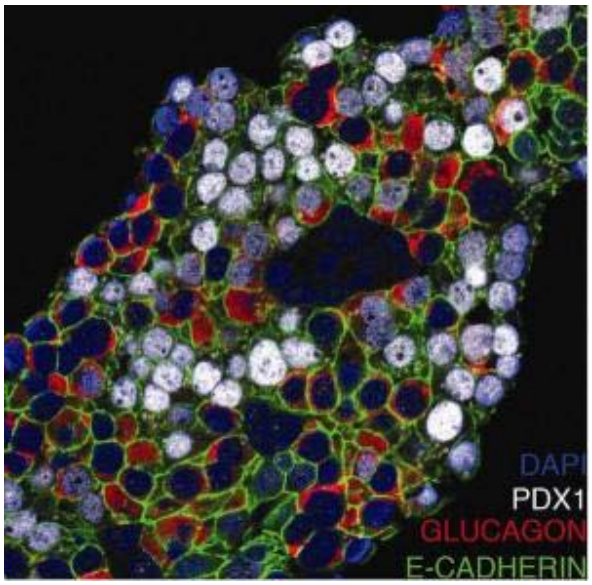
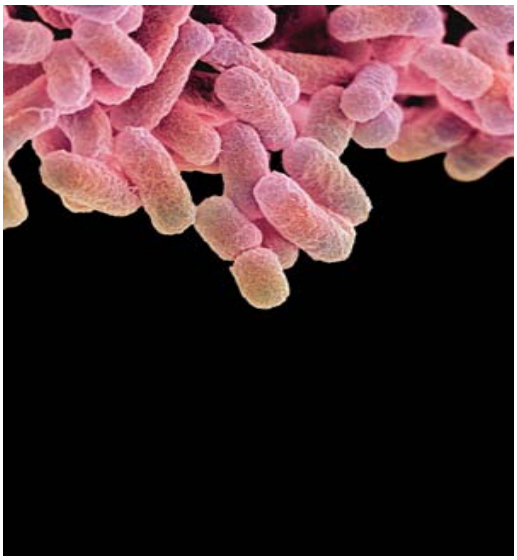


Figure: Treatment of human islets with the histone methyltransferase inhibitor Adox results in co-localization of the beta-cell specific transcription factor PDX1 (white) in a substantial sub-population of glucagon-positive cells (red), indicating partial endocrine cell-fate conversion.

GENETICALLY MODIFIED BACTERIA AND YEAST CAN MAKE GOLD, PHARMACEUTICAL COMPOUNDS AND FUELS.

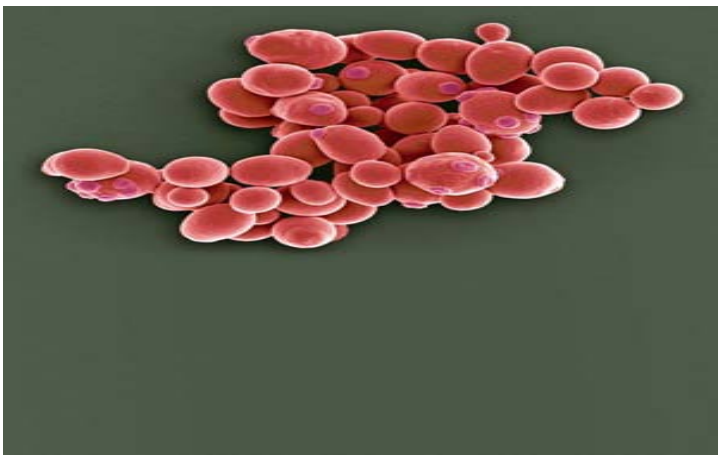
When Michigan State University artist Adam Brown learned of a type of bacteria, Cupriavidus metallidurans, that can extract pure gold from the toxic solution gold chloride (a totally artificial salt), he hurried to an expert colleague, microbiologist Kazem Kashefi, with a question: “Is it possible to make enough gold to put in the palm of my hand?” Brown merely wanted to satisfy his intellectual and artistic curiosity, inspired by the gold-tinted roots of alchemy, the precursor of modern chemistry. Soon thereafter, Kashefi and Brown set to work designing a half-experiment, half-art-exhibit that

exposes C. metallidurans to gold chloride in a hydrogen-gas-rich atmosphere that serves as a source of food. Over the course of a week, the bacteria gradually strip-mined the toxic liquid, leaving flecks of pure 24-karat gold behind. The inefficient technique won't supplant traditional mining, but the idea of using microbes as production facilities for a range of rare and difficult-to-produce materials has been gaining traction over the past several years.



OTHER MICROBIAL FACTORIES IN THE WORKS

Escherichia coli, or E. coli, is being used by University of Washington chemical engineer James Carothers to produce a chemical precursor of



pristinamycin, an antibiotic used to treat staph infections. The microbial method could reduce the chemical-processing steps usually necessary to make the drug, potentially lowering the cost. Real-world deployment is at least five years out.

Saccharomyces cerevisiae. Biologist Jay Keasling of the University of California in Berkeley has shown how this species of yeast can be engineered to produce artemisinic acid, a key chemical compound in anti-malaria drugs, normally extracted from the wormwood tree. Limited supply has driven up the cost of the drug, but now pharmaceutical giant Sanofi-Aventis has licensed the technology to use S. cerevisiae for mass production.

Methanobacterium palustre. Apply electric current to this microbe, and you produce pure methane, according to microbiologist Bruce Logan of Pennsylvania State University. Increasingly, methane is being mined through controversial methods like fracking, but with Logan's technique, we could achieve green production of methane gas to power our appliances and heat our homes. Across a range of applications, microbial factories just might introduce efficiencies that the Industrial Revolution never could achieve.

BEATING HEART CELLS IN A LAB DISH: CREATING NEW TISSUE INSTEAD OF TRANSPLANTING HEARTS

For the first time, scientists have successfully taken skin cells from heart failure patients and reprogrammed them into healthy, beating heart muscle cells that can integrate with surrounding tissue. It's likely that the procedure will need another decade of testing and fine-tuning, but researchers say the results of their study could mean a future of treating heart damage with a patient's own reprogrammed cells. In the current study, researchers in Haifa, Israel took skin cells from two male heart failure patients (ages 51 and 61) and transformed them by adding three genes and a small molecule called valproic acid to the cell nucleus. The team then mixed the newly formed cells with pre-existing heart tissue in the lab and watched as the cells began to beat together within a matter of days. In the final step of the experiment, the combined tissue was transplanted into rats, where it went on to integrate with the animals' surrounding heart cells. If the method is successful in humans, it could eliminate worries surrounding immune rejection since the cells would be a patient's own. The procedure would also avoid the ethical dilemmas often brought up by embryonic stem cell use since the reprogrammed stem cells do not use embryos.

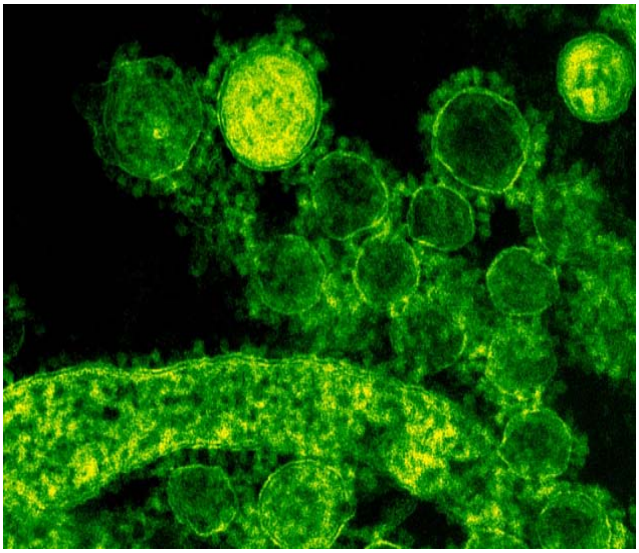
HUMAN ANTI-MICROBE PROTEIN ADDED TO GOAT MILK

Goat milk with extra lysozyme, an antimicrobial protein found in human breast milk, helps young pigs recover from diarrhea faster. The findings, published in PLOS ONE, offer hope that such milk may eventually help prevent human diarrheal diseases that each year claim the lives of 1.8 million children around the world and impair the physical and mental development of millions more.In this study, Murray and colleagues fed young pigs milk from goats that were genetically modified to produce in their milk higher levels of lysozyme, a protein that naturally occurs in the tears, saliva, and milk of all mammals.

Although lysozyme is produced at very high levels in human breast milk, the milk of goats and cows contains very little lysozyme, which prompted the effort to boost lysozyme levels in the milk of those animals using genetic modification.

RECEPTOR FOR NEW CORONAVIRUS FOUND

This week, researchers identified the molecule that has allowed a novel human coronavirus to infect at least 14 people, killing eight, since its detection last year. This key discovery, which



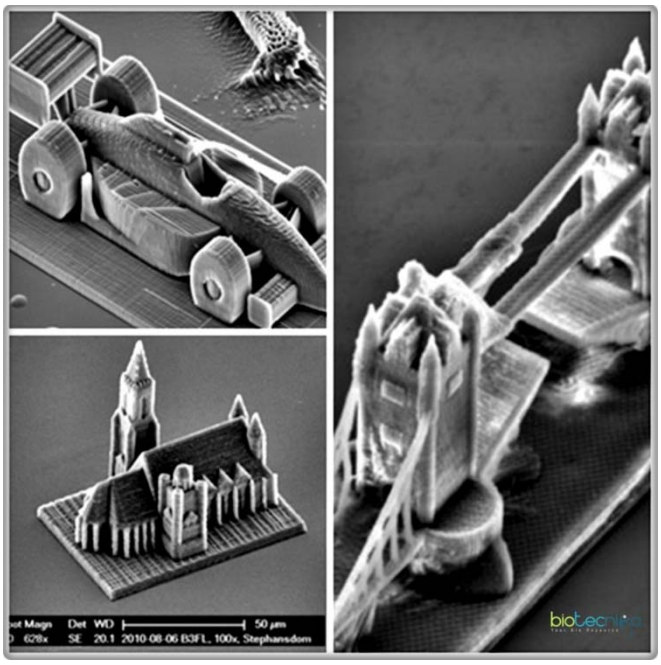
pinpoints the receptor that the virus uses to infect cells in the human airways, opens up opportunities to study the virus's origin, the level of risk it poses and potential drugs and vaccines. But it will take more than lab work to determine whether the virus is the next SARS — the coronavirus responsible for severe acute respiratory syndrome, which infected more than 8,000 people and killed more than 750 in the early 2000s — or just an exotic pathogen of little broad importance to public health. Only epidemiological data can show how efficiently the new coronavirus, hCoV-EMC, spreads from person to person and whether it is as deadly as it seems — such data are sorely lacking. To jump to humans, animal viruses such as these novel coronaviruses, and avian and swine flu viruses, must evolve to be able to latch onto proteins on the surfaces of human cells. In a paper published this week in Nature1, Stalin Raj at the Erasmus Medical Centre in Rotterdam, the Netherlands, and a largely European team report that spikes on the surface of hCoV-EMC bind to DPP4, a well-known receptor protein on human cells. When the binding site for the virus on DPP4 was blocked using antibodies, the virus could not infect cells; conversely, when DPP4 was expressed on the surface of normally non-susceptible cells, hCoV-EMC could now infect them.

GENE X3 HELPS CORN GROW IN ACIDIC SOIL

A genetic variation makes it possible for corn to grow in soil that contains high levels of aluminum, a chemical that is toxic to many plants. Identifying genes that make plants more tolerant of aluminum is critical for farmers growing crops where productivity is suboptimal due to acidic soil, researchers say. Approximately 30 percent of the world's total land is too acidic to support crop production, but certain strands of corn growing in tropical and subtropical areas have three copies of a particular gene that make them more tolerant. The triplicate gene may ultimately be used to breed or genetically modify plants to adapt to soil containing high levels of aluminum.

NANO-PRECISION 3D PRINTER

Printing three dimensional objects with incredibly fine details is now possible using "two-photon lithography". With this technology, tiny structures on a nanometer scale can be fabricated. Researchers at the Vienna University of Technology (TU Vienna) have now made a major breakthrough in speeding up this printing technique: The high-precision-3D-printer at TU Vienna is orders of magnitude faster than similar device. This opens up completely new areas of application, such as in medicine. The 3D printer uses a liquid resin, which is hardened at precisely the correct spots by a focused laser beam. The focal point of the laser beam is guided through the resin by movable mirrors and leaves behind a polymerized line of solid polymer, just a few hundred nanometers wide. This amazing progress was made possible by combining several new ideas. “It was crucial to improve the control mechanism of the mirrors”, says Jan Torgersen (TU Vienna). The mirrors are continuously in motion during the printing process. The acceleration and deceleration-periods have to be tuned very precisely to achieve high-resolution results at a record-breaking speed.



WOUND HEALING 'SWITCH' IDENTIFIED

Scientists from A*STAR's Institute of Medical Biology (IMB) have identified a molecular "switch" that controls the migration of skin cells necessary for wounds to close and heal. This is especially significant for diabetics and other patients who suffer from chronic wounds, wounds that do not heal or take years to do so, which are vulnerable to infections and could lead to amputations. This switch mechanism may hold the key to developing therapeutics that will reduce or prevent chronic wounds. The scientists discovered that a tiny "micro-RNA" molecule, called miR-198, controls several different processes that help wound healing, by keeping them switched off in healthy skin. When skin is wounded, the manufacture of miR-198 quickly stops and the levels of miR-198 drop, switching on many wound healing processes. In the non-healing wounds of diabetics, miR-198 does not disappear and wound healing remains blocked. This therefore identifies miR-198 as a potential diagnostic biomarker for non-healing wounds.

TURNING TOXIC BY-PRODUCT INTO BIO-FUEL BOOSTER - BIOTECH INNOVATION

Scientists studying an enzyme that naturally produces alkanes -- long carbon-chain molecules that could be a direct replacement for the hydrocarbons in gasoline -- have figured out why the natural reaction typically stops after three to five cycles. Armed with that knowledge, they've devised a strategy to keep the reaction going. The biochemical details -- worked out at the U.S. Department of Energy's Brookhaven National Laboratory and described in the Proceedings of the National Academy of Sciences the week of February 4, 2013 -- renew interest in using the enzyme in bacteria, algae, or plants to produce biofuels that need no further processing.

NANOPARTICLES LOADED WITH BEE VENOM KILL HIV

Nanoparticles carrying a toxin found in bee venom can destroy human immunodeficiency virus (HIV) while leaving surrounding cells unharmed, researchers at Washington University School of Medicine in St. Louis have shown. The finding is an important step toward developing a vaginal gel that may prevent the spread of HIV, the virus that causes AIDS. The study appears in the current issue of Antiviral Therapy. Bee venom contains a potent toxin called melittin that can poke holes in the protective envelope that surrounds HIV, and other viruses. Large amounts of free melittin can cause a lot of damage. Indeed, in addition to anti-viral therapy, the paper's senior author, Samuel A. Wickline, MD, the J. Russell Hornsby Professor of Biomedical Sciences, has shown melittin-loaded nanoparticles to be effective in killing tumor cells. The new study shows that melittin loaded onto these nanoparticles does not harm normal cells. That's because Hood added protective bumpers to the nanoparticle surface. When the nanoparticles come into contact with normal cells, which are much larger in size, the particles simply bounce off. HIV, on the other hand, is even smaller than the nanoparticle, so HIV fits between the bumpers and makes contact with the surface of the nanoparticle, where the bee toxin awaits.

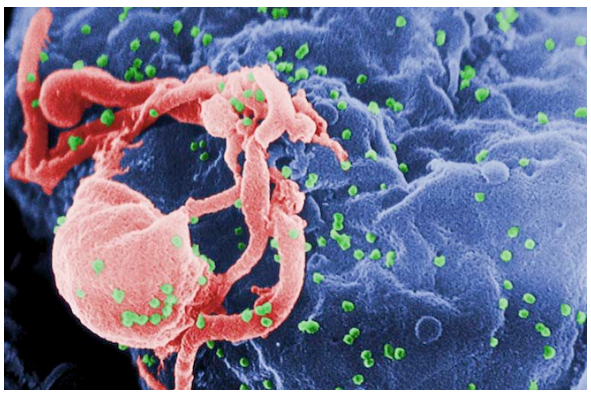


Figure: Nanoparticles (purple) carrying melittin (green) fuse with HIV (small circles with spiked outer ring), destroying the virus's protective envelope. Molecular bumpers (small red ovals) prevent the nanoparticles from harming the body's normal cells, which are much larger in size.

BIO-BATTERIES FROM BACTERIA

Scientists at the University of East Anglia have made an important breakthrough in the quest to generate clean electricity from bacteria. Findings published in the journal Proceedings of the National Academy of Sciences (PNAS) show that proteins on the surface of bacteria can produce an electric current by simply touching a mineral surface. The research shows that it is possible for bacteria to lie directly on the surface of a metal or mineral and transfer electrical charge through their cell membranes. This means that it is possible to 'tether' bacteria directly to electrodes – bringing scientists a step closer to creating efficient microbial fuel cells or 'bio-batteries'. *Shewanella oneidensis* (pictured) is part of a family of marine bacteria. The research team created a synthetic version of this bacteria using just the proteins thought to shuttle the electrons from the inside of the microbe to the rock. They inserted these proteins into the lipid layers of vesicles, which are small capsules of lipid membranes such as the ones that make up a bacterial membrane. Then they tested how well electrons travelled between an electron donor on the inside and an iron-bearing mineral on the outside.

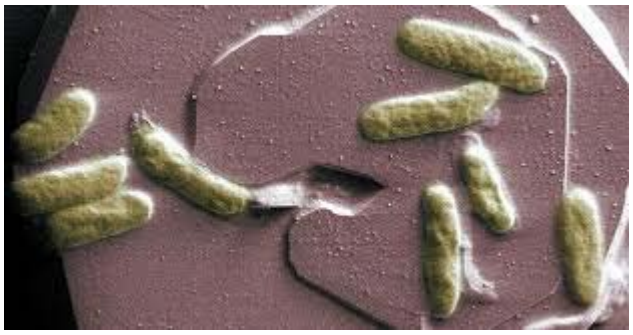
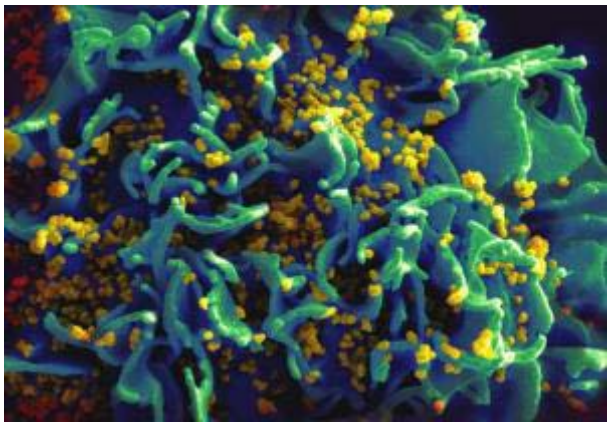


Figure: *Shewanella oneidensis*

CHILD BORN WITH HIV CURED : FIRST DOCUMENTED CASE FOR AIDS CURE

US doctors have managed the effective cure of a child born with the HIV virus. Dr. Deborah Persaud of Johns Hopkins University today described the first documented case of a child being cured of HIV. The landmark findings were announced at the 2013 Conference on Retroviruses and Opportunistic Infections in Atlanta, GA. Dr. Persaud, an amfAR grantee, detailed the case of a two-year-old child in Mississippi diagnosed with HIV at birth and immediately put on antiretroviral therapy. At 18 months, the child ceased taking antiretrovirals and was lost to follow-up. When brought back into care at 23 months, despite being off treatment for five months, the child was found to have an undetectable viral load. A battery of subsequent highly sensitive tests confirmed the absence



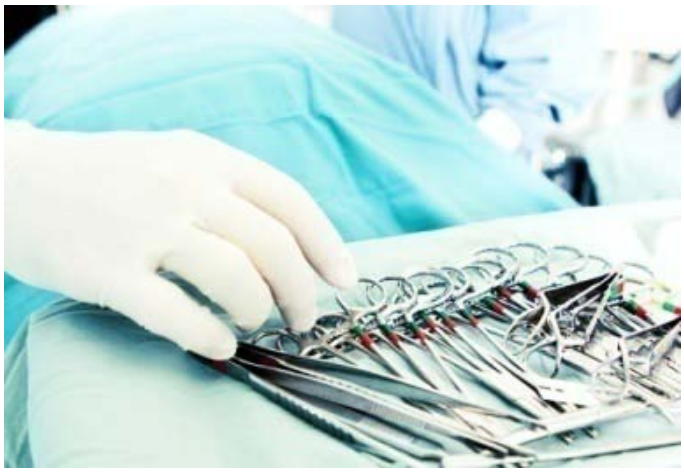
GENETICALLY MODIFIED TOBACCO PLANTS PRODUCE ANTIBODIES TO TREAT RABIES

New research in The FASEB Journal shows that transgenic tobacco plants can be used to produce safe, protective antibodies against rabies and to benefit patients in developing countries,

a researcher involved in the work from the Hotung Molecular Immunology Unit at St. George's, University of London**.** Smoking tobacco might be bad for your health, but a genetically altered version of the plant might provide a relatively inexpensive cure for the deadly rabies virus. This new antibody works by preventing the virus from attaching to nerve endings around the bite site and keeps the virus from traveling to the brain. To make this advance, Both and colleagues "humanized" the sequences for the antibody so people could tolerate it. Then, the antibody was produced using transgenic tobacco plants as an inexpensive production platform. The antibody was purified from the plant leaves and characterized with regards to its protein and sugar composition. The antibody was also shown to be active in neutralizing a broad panel of rabies viruses, and the exact antibody docking site on the viral envelope was identified using certain chimeric rabies viruses.

NEW TOOL PREDICTS BLOOD CLOTS AFTER SURGERY

Scientists have developed a more accurate way to determine which patients are at highest risk for blood clots in their legs or lungs after surgery. The researchers studied the medical histories of more than 470,000 surgical patients to determine which factors increased their risk of blood clots, also called venous thromboembolism (VTE). The team then created a nomogram, a type of calculator, which can help clinicians predict an individual's 30-day VTE risk. The results could change clinical practice by providing a more rational approach to preventing dangerous blood clots. Blood clots are a critical safety and quality challenge for hospitals around the nation. Although administering blood thinners, such as heparin, can prevent blood clots, these measures increase the risk of bleeding. To complicate matters, clinicians have had no way of determining which patients are at higher risk for blood clots, forcing them to adopt a one-size-fits-all approach to prevention. "The standard preventive measure is heparin," says study leader Robert Canter, an associate professor of surgery at University of California, Davis.

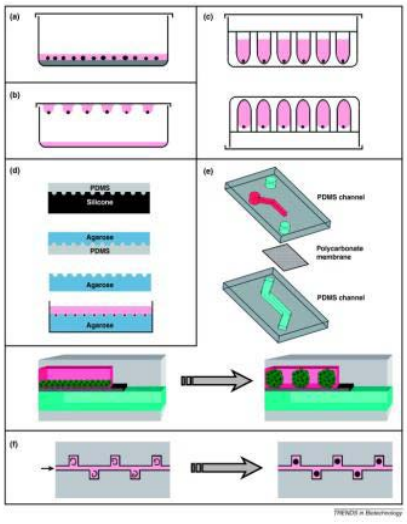


3D-PRINTED SKULL IMPLANTED IN AMERICAN PATIENT'S HEAD

Patient has new skull 'printed out' by scientists: American has 75 per cent of skull replaced with 3D-printed implant. There is no shortage of new and interesting uses for 3D printing technology. This week one more has been added to the list, and it's pretty darn impressive: replacing 75 percent of a patient's skull with a 3D-printed implant. The skull implant was approved by the FDA last month, and the surgery itself took place on March 4, as reported by Tech News Daily. The implant was made from a type of thermoplastic called polyetherketoneketone (PEKK). This material is moldable above a certain temperature, and returns to a solid state when it cools. Unlike most plastics, thermoplastics' long polymer chains do not break down during the melting process.

CELL THERAPY RESEARCH GROWS NERVE CELLS WITHIN BRAIN

The field of cell therapy, which aims to form new cells in the body in order to cure disease, has taken another important step in the development towards new treatments. A new report from researchers at Lund University in Sweden shows that it is possible to re-programme other cells to become nerve cells, directly in the brain. Two years ago, researchers in Lund were the first in the world to re-programme human skin cells, known as fibroblasts, to dopamine-producing nerve cells – without taking a detour via the stem cell stage. The research group has now gone a step further and shown that it is possible to re-programme both skin cells and support cells directly to nerve cells, in place in the brain. The researchers used genes designed to be activated or de-activated using a drug. The genes were inserted into two types of human cells: fibroblasts and glia cells – support cells that are naturally present in the brain. Once the researchers had transplanted the cells into the brains of rats, the genes were activated using a drug in the animals' drinking water. The cells then began their transformation into nerve cells.



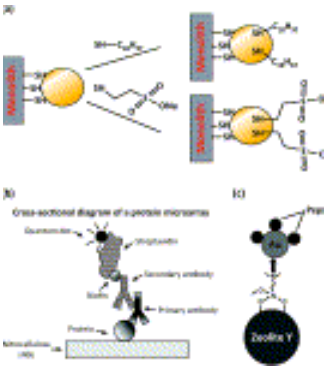
SPHEROID CULTURE AS A TOOL FOR CREATING 3D COMPLEX TISSUES

3D cell culture methods confer a high degree of clinical and biological relevance to *in vitro* models. This is specifically the case with the spheroid culture, where a small aggregate of cells grows free of foreign materials. In spheroid cultures, cells secrete the extracellular matrix (ECM) in which they reside, and they can interact with cells from their original microenvironment. The value of spheroid cultures is

increasing quickly due to novel microfabricated platforms amenable to high-throughput screening (HTS) and advances in cell culture. Here, we review new possibilities that combine the strengths of spheroid culture with new microenvironment fabrication methods that allow for the creation of large numbers of highly reproducible, complex tissues.

Figure: Spheroid fabrication methods: (a) liquid overlay technique; (b) hanging drop technique; (c) microwell hanging drop technique; (d) microwell array from micropatterned agarose wells; (e,f) microfluidic spheroid formation.

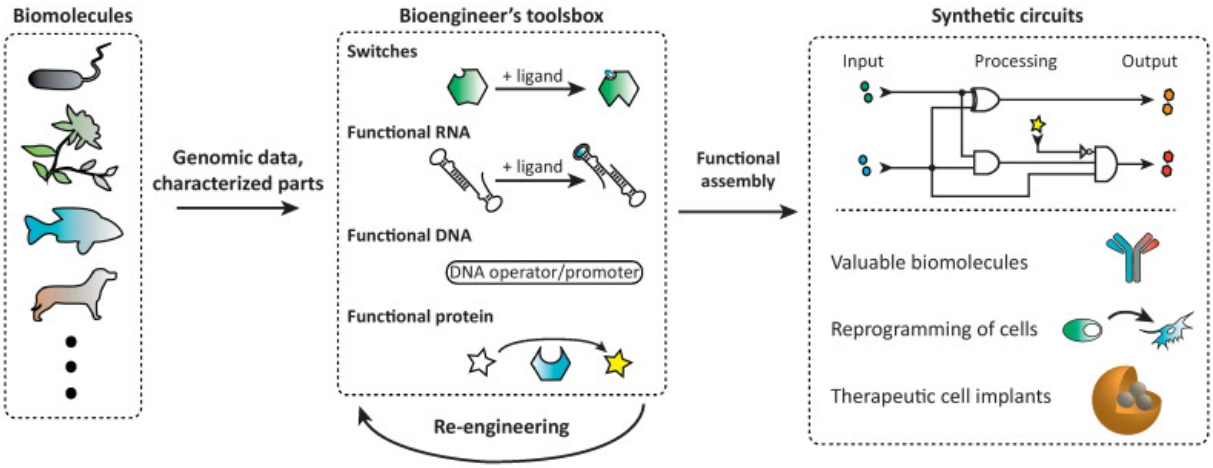
NANOPROTEOMICS



The complexity of proteomics challenges current methods to provide all peptide mass fingerprints in an *ensemble* measurement of various proteins at differing concentrations. To detect those low-abundance proteins, nanotechnology provides a technical platform to improve biocompatibility, specificity, reproducibility, and robustness of the current proteomic methods. The weaknesses of traditional proteomic methods and evaluate the importance of nanomaterials in significantly improving the quality of proteomic methods by manipulating individual proteins. We also illustrate how the large surface-to-volume ratio of nanomaterials can facilitate mass transfer, enhance the efficiency of separation and high-throughput capability, and reduce assay time and sample consumption. The marriage of the two subjects and the resulting new nanoproteomics will revolutionize proteomics research.

FROM GENE SWITCHES TO MAMMALIAN DESIGNER CELLS: PRESENT AND FUTURE PROSPECTS

Nature has evolved a treasury of biological molecules that are logically connected to networks,



enabling cells to maintain their functional integrity. Similar to electronic circuits, cells operate as information-processing systems that dynamically integrate and respond to distinct input signals. Synthetic biology aims to standardize and expand the natural toolbox of biological building blocks to engineer novel synthetic networks in living systems. Mammalian cells harboring

integrated designer circuits could work as living biocomputers that execute predictable metabolic and therapeutic functions.

SINGLE-USE DISPOSABLE TECHNOLOGIES FOR BIOPHARMACEUTICAL MANUFACTURING

The manufacture of protein biopharmaceuticals is conducted under current good manufacturing practice (cGMP) and involves multiple unit operations for upstream production and downstream purification. Until recently, production facilities relied on the use of relatively inflexible, hard-piped equipment including large stainless steel bioreactors and tanks to hold product intermediates and buffers. However, there is an increasing trend towards the adoption of single-use technologies across the manufacturing process. Technical advances have now made an end-to-end single-use manufacturing facility possible, but several aspects of single-use technology require further improvement and are continually evolving. This article provides a perspective on the current state-of-the-art in single-use technologies and highlights trends that will improve performance and increase the market penetration of disposable manufacturing in the future.

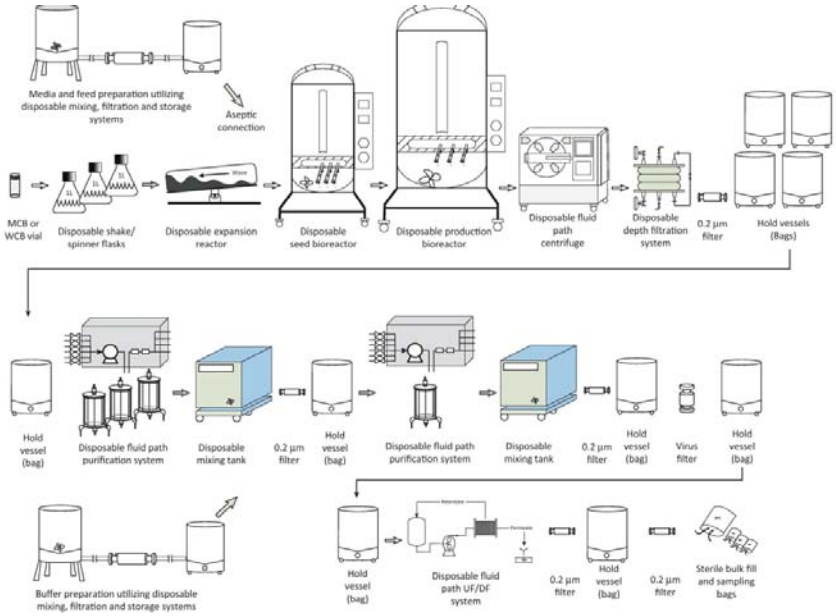


Figure: A biopharmaceutical drug substance production process.

FLOCCULATION AS A LOW-COST METHOD FOR HARVESTING MICROALGAE FOR BULK BIOMASS PRODUCTION

The global demand for biomass for food, feed, biofuels, and chemical production is expected to increase in the coming decades. Microalgae are a promising new source of biomass that may complement agricultural crops. Production of microalgae has so far been limited to high-value applications. In order to realize large-scale production of microalgae biomass for low-value applications, new low-cost technologies are needed to produce and process microalgae. A major challenge lies in the harvesting of the microalgae, which requires the separation of a low amount of biomass

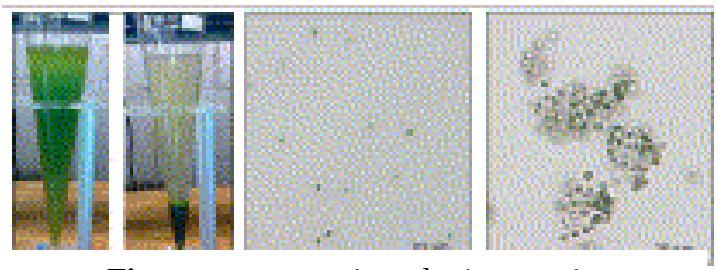


Figure: macroscopic and microscopic

consisting of small individual cells from a large volume of culture medium. Flocculation is seen as a promising low-cost harvesting method. Here, we overview the challenges and possible solutions for flocculating microalgae.

COMBINATION APPROACHES TO COMBAT MULTIDRUG-RESISTANT BACTERIA

The increasing prevalence of infections caused by multidrug-resistant bacteria is a global health problem that has been exacerbated by the dearth of novel classes of antibiotics entering the clinic over the past 40 years. Herein, we describe recent developments toward combination therapies for the treatment of multidrug-resistant bacterial infections. These efforts include antibiotic–antibiotic combinations, and the development of adjuvants that either directly target resistance mechanisms such as the inhibition of β-lactamase enzymes, or indirectly target resistance by interfering with bacterial signaling pathways such as two-component systems (TCSs).

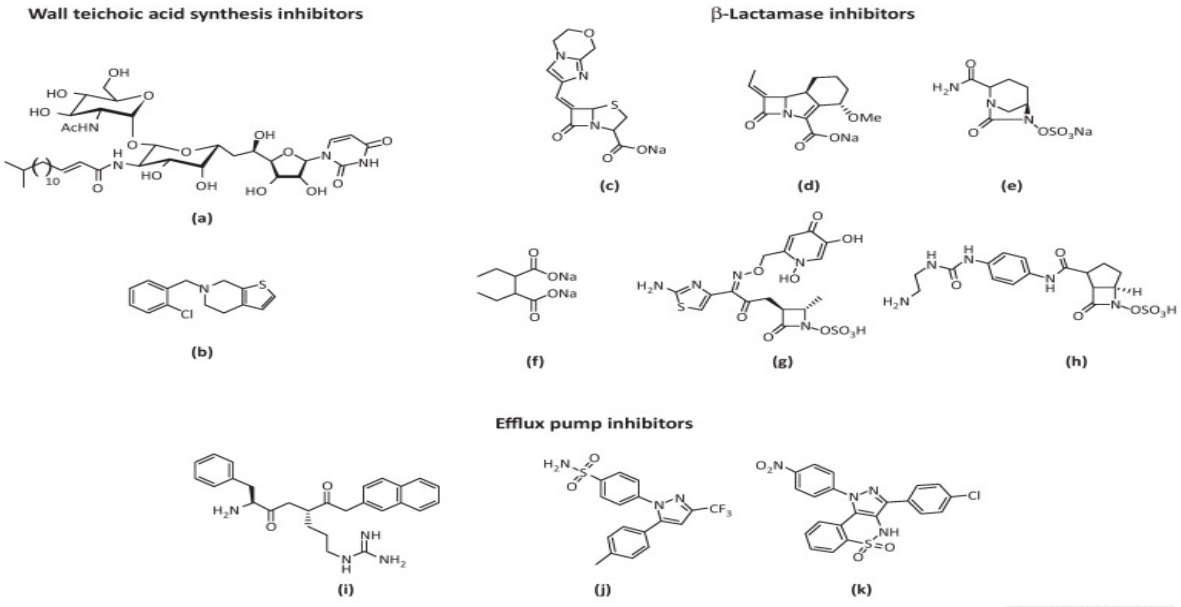
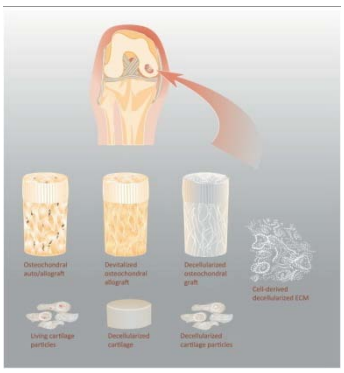


Figure: Combination therapy and traditional adjuvant targets

EXTRACELLULAR MATRIX SCAFFOLDS FOR CARTILAGE AND BONE REGENERATION



Regenerative medicine approaches based on decellularized extracellular matrix (ECM) scaffolds and tissues are rapidly expanding. The rationale for using ECM as a natural biomaterial is the presence of bioactive molecules that drive tissue homeostasis and regeneration. Moreover, appropriately prepared ECM is biodegradable and does not elicit adverse immune responses. Successful clinical application of decellularized tissues has been reported in cardiovascular, gastrointestinal, and breast reconstructive surgery. At present, the use of ECM for

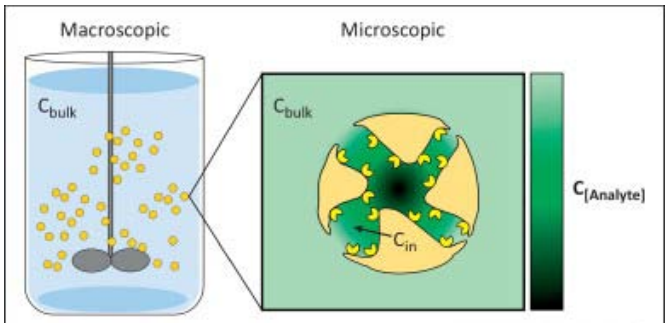
osteochondral tissue engineering is attracting interest. Recent data underscore the great promise for future application of decellularized ECM for osteochondral repair.

IMMOBILIZED ENZYMES: REAL-TIME SENSING IN SOLID SUPPORTED BIOCATALYSTS

Enzyme immobilization on solid supports has been key to biotransformation development. Although technologies for immobilization have largely reached maturity, the resulting biocatalysts are not well understood mechanistically. One limitation is that their internal environment is usually inferred from external data. Therefore, biological consequences of the immobilization remain masked by physical effects of mass transfer, obstructing further development. Work reviewed herein shows that opto-chemical sensing performed directly within the solid support enables the biocatalyst's internal environment to be uncovered quantitatively and in real time. Non-invasive methods of intraparticle pH and O_2 determination are presented, and their use as process analytical tools for development of heterogeneous biocatalysts is described. Method diversification to other analytes remains a challenging task for the future.

MINERAL CO_2 SEQUESTRATION BY ENVIRONMENTAL BIOTECHNOLOGICAL PROCESSES

CO_2 sequestration may be an avenue to mitigate climate change. CO_2 sequestration by mineral carbonation can be achieved by the reaction of CO_2 with alkaline silicates. Here, we evaluate how alkaline silicate mineral-based CO_2 sequestration can be achieved using environmental biotechnological processes. Several biotechnological processes rely on the sequence of (i) an acid-producing reaction such as nitrification and anaerobic fermentation and (ii) an alkalinity-producing reaction such as denitrification and methanogenesis. Whereas the acid-producing reaction can be used to enhance the dissolution of, for example, alkaline calcium silicates, the subsequent alkalinity-producing step can precipitate $CaCO_3$. We quantitatively evaluate the potential of these processes for CO_2 sequestration and propose that optimization of these processes could contribute to climate change mitigation strategies.



STEM CELL METABOLIC AND SPECTROSCOPIC PROFILING

Stem cells offer great potential for regenerative medicine because they regenerate damaged tissue by cell replacement and/or by stimulating endogenous repair mechanisms. Although stem cells are defined by their functional properties, such as the potential to proliferate, to self-renew, and to differentiate into specific cell types, their identification based on the expression of specific markers remains vague. Here, profiles of stem cell metabolism might highlight stem cell function more than the expression of single genes/markers. Thus, systematic approaches including

spectroscopy might yield insight into stem cell function, identity, and stemness. We review the findings gained by means of metabolic and spectroscopic profiling methodologies, for example, nuclear magnetic resonance spectroscopy (NMRS), mass spectrometry (MS), and Raman spectroscopy (RS), with a focus on neural stem cells and neurogenesis.

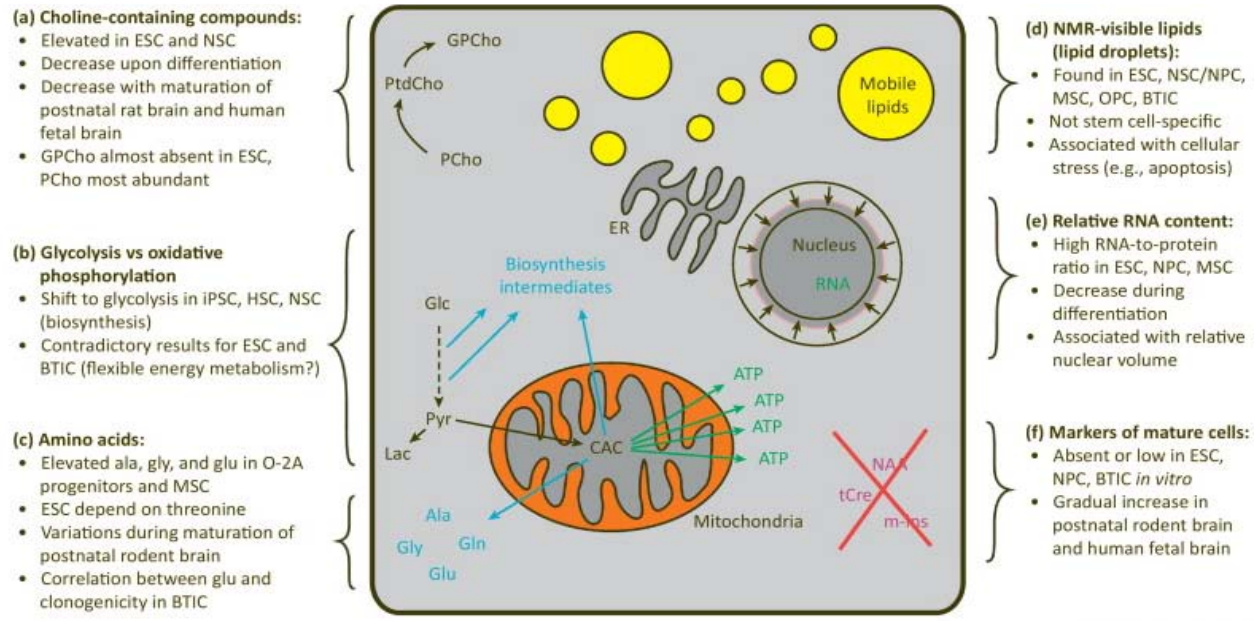
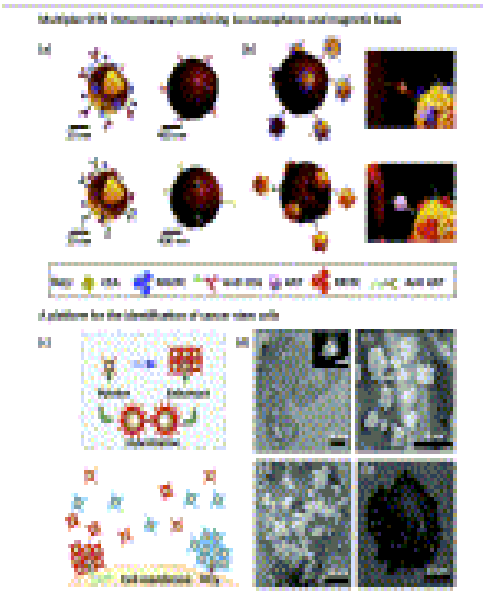


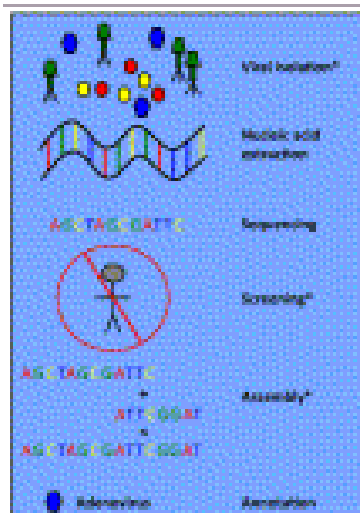
Figure: Major stem cell-specific findings of metabolic profiling approaches

SURFACE-ENHANCED RAMAN SCATTERING



Technologies that use surface-enhanced Raman scattering (SERS) have experienced significant growth in biomedical research during the past 4 years. In this review we summarize the progress in SERS for cancer diagnostics, including multiplexed detection and identification of new biomarkers, single-nucleotide polymorphisms, and circulating tumor cells. SERS is also used as a non-invasive tool for cancer imaging with immunoSERS microscopy, histological analysis of biopsies, and *in vivo* detection of tumors. We discuss the future of SERS probes compatible with multiple imaging modalities and their potential for clinical translation (e.g., endoscope-based and intraoperative imaging as tools for surgical guidance). Moreover, we highlight the potential of SERS agents for targeted drug delivery and photothermal therapy.

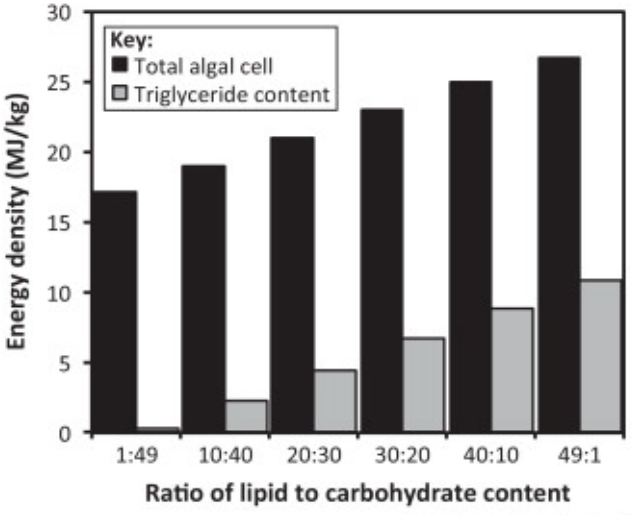
METAGENOMIC IDENTIFICATION OF VIRAL PATHOGENS



The target-independent identification of viral pathogens using 'shotgun' metagenomic sequencing is an emerging approach with potentially wide applications in clinical diagnostics, public health monitoring, and viral discovery. In this approach, all viral nucleic acids present in a sample are sequenced in a random, shotgun manner. Pathogens are then identified without the prerequisite of searching for a specific viral pathogen. In this opinion article, I discuss the current state and future research directions for this emerging and disruptive technology. With further technical developments, viral metagenomics has the potential to be deployed as a powerful and widely adopted tool, transforming the way that viral disease is researched, monitored, and treated.

NITROGEN SUPPLY IS AN IMPORTANT DRIVER OF SUSTAINABLE MICROALGAE BIOFUEL PRODUCTION

Favorable growth characteristics continue to generate interest in using triacylglycerides (TAGs) produced from microalgae for biodiesel feedstocks. Due to the energy consumption associated with the production of external nitrogen fertilizers, the manner in which nitrogen is supplied to microalgae biorefineries will be an important driver of energy yields, sustainability, and commercial success. Schemes including the reuse of urban wastewater represent improvements on the overall energy balance, but will not allow for significant production of biofuels unless the nitrogen from the non-TAG portions of microalgae is recycled. Approaches to recycling nitrogen require an improved understanding of the tradeoffs between the different potential uses of the non-TAG microalgal portion (i.e., energy production via anaerobic digestion or thermal catalytic processes), and the development of nitrogen separation technologies.



SYNTHETIC BIOLOGY, THE BIOECONOMY, AND A SOCIETAL QUANDARY

Opinions on what synthetic biology actually is range from a natural extension of genetic engineering to a new manufacturing paradigm. It offers, for the first time in the life sciences, rational design and engineering standardisation. It could address problems across a broad spectrum of human concerns, including energy and food security, and health of growing and aging populations. It also offers great scope for public resistance to its introduction to daily life.

Development of Pineapple Sector in Kerala in Mission Mode

Project Proposal under Pineapple Mission

Submitted to

Sri. K.P. Mohanan
Hon. Minister for Agriculture
Government of Kerala

by

Dr. P. P. Joy
Associate Professor & Head

KERALA AGRICULTURAL UNIVERSITY
PINEAPPLE RESEARCH STATION
Vazhakulam-686 670, Muvattupuzha, Ernakulam District, Kerala
Tel. & Fax: 0485-2260832, Mobile: 9446010905
Email: prsvkm@kau.in, prsvkm@gmail.com
Web: www.kau.edu/prsvkm, http://prsvkm.tripod.com

28.07.2012

PROJECT PROPOSAL UNDER PINEAPPLE MISSION

1. Title of the project : Development of Pineapple Sector in Kerala in Mission Mode

2. Project Officer : Dr. P. P. Joy, Associate Professor & Head, Pineapple Research Station (Kerala Agricultural University), Vazhakulam, Muvattupuzha, Ernakulam PIN-686 670, Tel. & Fax: 0485-2260832, Cell: +919446010905
E-mail: prsvkm@kau.in, prsvkm@gmail.com
Website: www.kau.edu/prsvkm, http://prsvkm.tripod.com

3. Background

Pineapple is an important tropical fruit showing an increasing demand world wide, over the years. World trade on fresh pineapple has shown 100 % increase during the last one decade. Even though India is the fifth largest producer of pineapple in the world with a production of 14,15,400 tonnes from an area of 88,700 hectares, its share in the world market is only 0.1 %. The different Asian countries and the countries around the Indian ocean are importing about two lakh tonnes of pineapple in a year, mostly coming from distant countries. This market can be exploited by Kerala if an earnest effort is made in the right direction.

In Kerala, pineapple is grown mainly as an intercrop in rubber and coconut plantations, as pure crop in garden land and in converted paddy fields. Pineapple fruits are produced round the year in Kerala. It is grown in 10,200 hectares producing 85,500 tonnes annually with a productivity of 8.4 t/ha as compared to the all India average of 16 t/ha. Kerala contributes only 6% to national production though it occupies 11.5% in area. There is great scope for productivity improvement through adoption of Kerala Agricultural University Package of Practices recommendations for pineapple (*Ananas comosus* var. Mauritius).

There is immense potential to increase the area under pineapple in Kerala as it can be grown as an intercrop in coconut and rubber plantations. Coconut is grown in about 7,78,618 hectares and if pineapple is grown as an intercrop in coconut plantations it will give an additional income, especially in root wilt affected areas it will be a solace to the farmers. Special attention can be given for intercropping pineapple in coconut in root wilt affected areas.

Out of the 5,25,408 hectares of rubber grown, about 15,000 hectare is replanted every year. Pineapple is grown as catch crop for the first three years in rubber at the time of replanting. Pineapple cultivation in rubber will give income to farmers during the period when there is no income from rubber. However, only less than two percent of the potential area in Kerala is cultivated with pineapple. At present pineapple cultivation in Kerala is generating employment of about 45 lakh mandays among farmers, agricultural workers, people involved in loading, unloading, transporting, traders, retailers etc. By doubling the area under pineapple cultivation, an additional 45 lakhs work days per year can be created.

The pineapple cultivation in Kerala is dependent on fresh fruit market, supplying most of its produce to outside Kerala. The Mauritius variety grown in Kerala is marketed in about 10

states in India including New Delhi. It is also exported to Gulf countries in limited quantities. It is possible to increase its marketing by exploring new markets and techniques and also by increase in quality and quantity of fruit produced. It is essential to explore the possibility for marine exports to reduce cost. Consumer preference and marketing strategies are to be taken into consideration. The annual value of pineapple produced in Kerala comes to about 350 crores which may be next to cardamom and coffee. But it is doubtful whether it is getting sufficient attention it deserves.

The Pineapple Research Station at Vazhakulam was established on 1995 to give research and development support to the pineapple growers, provide quality technology, products and services to the pineapple sector and undertake basic and applied research in pineapple and other fruit crops of Kerala. This centre of Kerala Agricultural University has taken up research in pineapple on various aspects, identified pineapple and passion fruit varieties suitable for Kerala, developed scientific and organic production technologies for Kew and Mauritius varieties of pineapple in pure cropping, intercropping in rubber and coconut plantations and in paddy lands. It also developed tissue culture protocols for various varieties of pineapple, passion fruit and banana. Based on continuous surveillance and laboratory studies the station has identified the presence of PMWA virus in pineapple in Vazhakulam area. Based on all the findings this station has formulated the Package of Practices Recommendations for the popular variety Mauritius and included in the POP and all the technology developed are being delivered to the pineapple growers extensively. The GI registration for Vazhakulam pineapple has improved its export.

There is immense potentiality of boosting pineapple production in Kerala. The region has all sets of climate and sufficient rainfall for growing pineapple very successfully. There is a great possibility of expanding its cultivation to the low and mid lands of the state on a commercial scale. Pineapple in processed form like concentrates and RTS has tremendous scope of market both at national and international level because of its unique and excellent flavour and aroma. Pineapple having numerous medicinal properties has great scope for domestic and export market. Pineapple industry can bring about a much needed employment opportunity in the region with not only its cultivation but also with setting up of more processing and semi-processing units in the state. A concentrated effort on the other hand is much needed in creating awareness, developing market linkages, setting up of semi processing & large scale processing units, post harvest management, creation of proper infrastructures and logistics. Growing and marketing of export quality pineapple would play a big role in poverty alleviation through income generation, employment creation and health improvement. The above strategies would certainly boost the pineapple cultivation in the state.

4. Objective

To boost the production and productivity of superior quality GI registered Vazhakulam pineapple in Kerala through comprehensive multi-pronged integrated approach in mission mode

5. Technical programme

The project in mission mode encompasses a number of modules like Front Line Demonstrations on Pineapple Cultivation; Augmentation of Tissue Culture Production; Establishment of Processing Lab; Quality Assurance in Pineapple Production, Processing and Value addition; Training on Pineapple Cultivation, Processing and Value addition; Publications on pineapple in the form of leaflets, booklets, books, etc; Development of multimedia CDs and DVDs on pineapple cultivation, processing, etc and Development of public friendly website on pineapple with farmers' participatory approach involving Vegetable and Fruit Promotion Council of Kerala, Pineapple Farmers' Association, etc in Kerala to boost the production and productivity of superior quality GI registered Vazhakulam pineapple in Kerala.

i) Front Line Demonstrations on Pineapple Cultivation

Pineapple production can be increased through area expansion and productivity improvement. The average productivity in Kerala is only 8.2 t/ha as compared to the all India average of 16 t/ha. Adoption of Kerala Agricultural University Package of Practices recommendations for pineapple (*Ananas comosus* var. Mauritius) is expected to produce 25-30 t/ha under intercropping and 40-45 t/ha under pure cropping. Front Line demonstrations of this technology in research stations and farmers' fields will go a long way in improving the productivity.

Front Line Demonstration area: 0.2 ha (0.5 Acre)
Number of Front Line Demonstrations: 16 (1 in Research Station field, 15 in the farmers' fields of VFPCK or Pineapple Farmers' Association, covering Ernakulam, Idukki and Kottayam districts)

Front Line Demonstrations will be executed and supervised by the project staff with farmers participation. Input cost and labour charges will be provided @ ₹35000 in first year and ₹20000 in second and third years per demonstration. A vehicle is also required for execution and supervision of the Front Line Demonstrations, besides procurement of various Farm implements, Tools, etc.

Cost Estimates (₹ Lakh)

No	Item	I Year	II Year	III Year	Total
1	Project Fellow, B.Sc.(Agri./Hort.)/ M.Sc. 2Nos.@15000/Month	3.6	3.6	3.6	10.8
2	Recurring Contingencies	7.0	4.0	4.0	15.0
3	Non Recurring Contingencies (Vehicle, Farm implements, Tools)	10.0			10.0
	Total	**20.6**	**7.6**	**7.6**	35.8

ii) Augmentation of Tissue Culture Production

Availability of planting material in time and in adequate quantities is to be ensured for boosting pineapple production. Mass production of planting material is possible through tissue culture. Virus diseases are very serious problems in pineapple curtailing productivity. Healthy virus free plants can be selected through virus indexing technique. Virus indexing protocols will be developed for various varieties of pineapple to mass produce virus free planting materials. Once the virus indexing and tissue culture protocols are developed optimising media, incubation and hardening, mass production of various varieties of pineapple will be undertaken depending upon the demand from growers and industry. The various varieties of pineapple produced in large scale through tissue culture will be distributed to growers and other needy groups for promoting large scale cultivation in Kerala.

This requires standardisation of the protocols and augmentation of tissue culture production facilities at the centre, like renovation of building, procurement of various TC Lab Equipments, Laminar Air Flow cabinets, TC racks, UPS, etc.

Cost Estimates (₹ Lakh)

No	Item	I Year	II Year	III Year	Total
1	Project Fellows, M.Sc.(Biotech & Microbiology), 2Nos.@15000/Month	3.6	3.6	3.6	10.8
2	Recurring Contingencies	2.0	2.5	3.0	7.5
3	Non Recurring Contingencies (TC Lab Equipments, TC racks, UPS, etc)	5.0	2.0		7.0
	Total	**10.6**	**8.1**	**6.6**	25.3

iii) Establishment of Processing Lab

There is great uncertainty in the market price of pineapple due to the interplay of demand and supply. Market price fluctuates between ₹5 and ₹40 due to reasons beyond control. If small home scale fruit processing units are started in the hot spots of pineapple production, the market fluctuation can be reduced and substantial employment generation is effected especially for the rural youth and women. Procurement in seasons of plenty or market glut and conversion into juice concentrates for later manufacture of value added products stabilises market price considerably. Establishment of a fruit processing laboratory at PRS, Vazhakulam for the efficient conversion of leftover fruits to value added products like squash, jam, syrup, etc will also cater to function as a demonstration and training unit for the various self-help groups in the state, besides fetching additional revenue for the station. Hence it is essential to design, set up and manage a world class Fruit Processing lab with FPO registration at the centre, develop novel recipes and fruit products and undertake regular Fruit Processing tasks.

The existing building has to be renovated with floor and wall tiles, conforming to the guidelines for FPO Registration. Doors, windows and exhausts have to be secured with wire nets to make the unit insect proof. Essential equipment, furniture, storage cans, bottles utensils, tools,

raw materials, etc have to be procured for various activities such as juice extraction, pulp production, heating, sterilisation and preparation of squash, jam, syrup, etc. FPO Registration has to be obtained for marketing of the finished products. A vehicle is also required for transportation of raw materials from farm and finished products.

Cost Estimates (₹ Lakh)

No	Item	I Year	II Year	III Year	Total
1	Project Fellow, M.Sc.(Processing/Food.Tech/Home Science), 1No.@15000/Month	1.8	1.8	1.8	5.4
2	Recurring Contingencies	2.0	2.5	3.0	7.5
3	Non Recurring Contingencies (Renovation, Processing Equipments, Tools, Utensils as per FPO guidlines)	7.0	2.0	-	9.0
	Total	**10.8**	**6.3**	**4.8**	21.9

iv) Quality Assurance in Pineapple Production, Processing and Value addition

Quality assurance has great significance especially when it comes to marketing and export. Lab facilities have to be created for the quality analyses of fruits such as estimation of moisture, pH, acidity, reducing, non-reducing & total sugar, TSS, protein, fibre, fat, minerals, vitamins, amino acids, enzymes, residues of ethephon, pesticides, heavy metals, etc.

Cost Estimates (₹ Lakh)

No	Item	I Year	II Year	III Year	Total
1	Project Fellow, M.Sc.(Agri./Hort.)/ /Biochem/Processing/Food.Tech/ Home Science), 1No.@15000/Month	1.8	1.8	1.8	5.4
2	Recurring Contingencies	1.0	1.5	2.0	4.5
3	Non Recurring Contingencies (Lab Equipments, Computer, Accessories)	5.0	2.0		7.0
	Total	**7.8**	**5.3**	**3.8**	16.9

v) Training on Pineapple Cultivation, Processing and Value addition

Developing suitable varieties and agro-technology and enhancing participatory dissemination of agricultural technologies and information to the farming community with the overall aims of improving food security, reducing poverty and contributing to more sustainable natural resource management will go a long way in popularizing pineapple. Developing various marketing channels and organized marketing will pave the way to commercial cultivation of the crop in Kerala. Further, group training is a suitable way of up-scaling technology dissemination to ensure technology adoption and sustainability. A concentrated effort, through training is much

needed in creating public awareness about the nutrition and health benefits of pineapple, need for growing improved varieties adopting scientific cultivation technology, post harvest management, onfarm processing and value addition, developing market linkages, setting up of semi processing & large scale processing units and creation of proper infrastructures and logistics. The above strategies would certainly boost the pineapple cultivation and productivity in the state. Adequate infrastructure and training facilities have to be developed for the purpose. Two to three days stipendiary trainings of farmers, self-help groups, extension personnel, etc in batches of 20-25 are very effective for dissemination of technology to all concerned.

Cost Estimates (₹ Lakh)

No	Item	I Year	II Year	III Year	Total
1	Project Fellow, B.Sc.(Agri./Hort.)/ M.Sc.(Processing/Food.Tech/Home Sci/Biochem), 1Nos.@15000/Month	1.8	1.8	1.8	5.4
2	Recurring Contingencies	1.0	1.3	1.5	3.8
3	Non Recurring Contingencies (Photocopier, Table, Chair, Almirah)	1.5	-	-	1.5
	Total	**4.3**	**3.1**	**3.3**	10.7

vi) Publications on pineapple in the form of leaflets, booklets, books, etc

Intense efforts to bring out as many publications on pineapple in the form of leaflets, booklets, books, etc, both in Malayalam and English will go a long way in creating public awareness about the nutrition and health benefits of pineapple, need for growing improved varieties adopting scientific cultivation technology, post harvest management, onfarm processing and value addition, developing market linkages, setting up of semi processing & large scale processing units, creation of proper infrastructures and logistics. This strategy would certainly transfer the technology easily, efficiently and precisely and boost the pineapple cultivation and productivity in the state. Adequate infrastructure and publishing facilities have to be developed for the purpose.

Cost Estimates (₹ Lakh)

No	Item	I Year	II Year	III Year	Total
1	Project Fellow M.Sc.(DTP/IT/Allied Science), 1No.@15000/Month	1.8	1.8	1.8	5.4
2	Recurring Contingencies	0.5	0.1	2.0	2.6
3	Non Recurring Contingencies (Camera, Computer, Accessories, etc)	2.0			2.0
	Total	**4.3**	**1.9**	**3.8**	10.0

vii) Development of multimedia CDs and DVDs on pineapple cultivation, processing, etc

Science is advancing at break-neck speed and this is the digital era. Unless we adapt to and progress in the digital technology we will be left out in the scientific revolution and remain ashamed in the primitive age. Intense efforts to create as much information in digital form with multimedia capabilities in the form of multimedia CDs and DVDs on pineapple cultivation, processing, etc, both in Malayalam and English will go a long way in creating public awareness about the nutrition and health benefits of pineapple, need for growing improved varieties adopting scientific cultivation technology, post harvest management, onfarm processing and value addition, developing market linkages, setting up of semi processing & large scale processing units. This strategy would certainly transfer the technology easily, efficiently and precisely and boost the pineapple cultivation and productivity in the state. Adequate infrastructure facilities have to be developed for the purpose.

Cost Estimates (₹ Lakh)

No	Item	I Year	II Year	III Year	Total
1	Project Fellow M.Sc.(IT/Multimedia Prodn/Allied), 1No.@15000/Month	1.8	1.8	1.8	5.4
2	Recurring Contingencies	0.5	0.7	1.0	2.2
3	Non Recurring Contingencies	1.0	-	-	1.0
	Total	**3.3**	**2.5**	**2.8**	8.6

viii) Development of public friendly website on pineapple

Information revolution is taking place in real time. In this era information is only a click/touch away. Easy access to information in real time is the need of the hour. Hosting the information in web servers, both in Malayalam and English will go a long way in creating public awareness about the nutrition and health benefits of pineapple, need for growing improved varieties adopting scientific cultivation technology, post harvest management, onfarm processing and value addition, developing market linkages, setting up of semi processing & large scale processing units. This is especially important for the educated public in Kerala, where the IT literacy is highest. It is more so in pineapple cultivation which has already attained agribusiness status in Kerala. This strategy would certainly transfer the technology to the educated public easily, efficiently and precisely and boost the pineapple cultivation and productivity in the state. Adequate infrastructure and web hosting facilities have to be developed for the purpose.

Cost Estimates (₹ Lakh)

No	Item	I Year	II Year	III Year	Total
1	Project Fellow M.Sc.(Web Tech/IT/ Multimedia/Allied), 1No.@15000/Month	1.8	1.8	1.8	5.4
2	Recurring Contingencies	0.5	0.7	1.0	2.2
3	Non Recurring Contingencies	1.0	-	-	1.0
	Total	**3.3**	**2.5**	**2.8**	8.6

6. Project Period: Three years because the economic life span is 3 years for Mauritius pineapple.

7. Expected Outcome

Pineapple fruits are made use of in three segments primarily. They are (i) Fresh Pineapple for internal market and export (ii) Canned pineapple (iii) Pineapple juice concentrate. Desirable fruit characteristics for the above mentioned segments are the following.

Fresh Pineapple for internal market and export	***Pineapple for canning industry***	***Pineapple for juice extraction & concentrates***
Pleasant flavour	Broad, flat fruit lets	Homogenous ripening fruit
High sugar content	Flesh, slightly translucent when ripe	High sugar content (18 - 20^0 brix)
Moderate acidity	Small core	Juicy fruit
Firm epidermis	Cylindrical in shape	Less fibre content
Short peduncle	Fruit weight: 1.5 kg	Fruit weight: 1.5 Kg – 1.8 kg
Excellent post harvest shelf life, should retain green skin for a long period even after harvest.		
Should withstand cold storage temperature 8 - 12^0 without internal browning		
Fruit weight: 0.9 – 1.2 kg		

Effective and timely implementation of the project will boost the pineapple production and availability for each of the user segments, namely, for internal market and export, canning industry and juice extraction and concentrates. It is expected to produce 30-50 thousand tissue culture plants annually and over one lakh tissue culture plants by the end of the project period. As a result of mass production of good quality tissue culture plants and large scale distribution of planting materials to the growers, industry and other needy groups the cultivation of pineapple will be boosted. Front Line demonstrations will convince all concerned, about the possible boosting of productivity with the presently available comprehensive integrated technology. There will be area expansion in the low and mid lands of the state on a commercial scale. Establishment of processing lab with FPO registration as a demonstration cum training unit will boost the home-scale, pilot-scale and commercial utilisation of pineapple. Technology dissemination through training and publications like leaflets, booklets, books, CDs, DVDs, etc

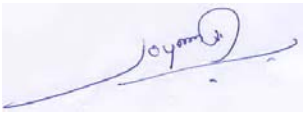
will become easy, efficient and precise, invoking better adoption. Real-time access to technology and information will ensure success of the pineapple agribusiness not only in the state but globally at large.

Area expansion coupled with productivity improvement will boost the pineapple industry increasing employment opportunity in the region with not only its cultivation but also setting up of more processing and semi-processing units in the state. Timely and planned market interventions by all concerned will stabilise the market price of pineapple considerably. A concentrated effort on the other hand is much needed in creating awareness, developing market linkages and setting up of semi processing & large scale processing units, post harvest management and creation of proper infrastructures and logistics. Growing and marketing of export quality pineapple would play a big role in poverty alleviation through income generation, employment creation and health improvement.

8. Budget (₹ Lakh)

Sl.No	Item	I Year	II Year	III Year	Total
1.	Front Line Demonstrations on Pineapple Cultivation	20.6	7.6	7.6	**35.8**
2.	Augmentation of Tissue Culture Production	10.6	8.1	6.6	**25.3**
3.	Establishment of Processing Lab	10.8	6.3	4.8	**21.9**
4.	Quality Assurance in Pineapple Production, Processing and Value addition	7.8	5.3	3.8	**16.9**
5.	Training on Pineapple Cultivation, Processing and Value addition	4.3	3.1	3.3	**10.7**
6.	Publications on pineapple in the form of leaflets, booklets, books	4.3	1.9	3.8	**10.0**
7.	Development of multimedia CDs and DVDs on pineapple cultivation, processing, etc	3.3	2.5	2.8	**8.6**
8.	Development of public friendly website on pineapple	3.3	2.5	2.8	**8.6**
	Total	**65.0**	**37.3**	**35.5**	**137.8**

(Rupees one crore thirty seven lakh eighty thousand only)



Vazhakulam,
28 July 2012

Dr. Joy P.P.
Head & Project Officer
Pineapple Research Station, Vazhakulam

7.10 Bill var expenditure details of Pineapple Research Station, Vazhakulam for 2012-13

321 Pineapple Research Station

321-31-0034 Non-Plan

BR No	110	120	130	142	152	210	222	223	236	237	300	330	410	418	420	821	840	Total
1/12-13	92143																	**92143**
2/12-13			22494															**22494**
7/12-13			22494															**22494**
14/12-13	92143																	**92143**
23/12-13			22494															**22494**
25/12-13			22494															**22494**
31/12-13	92143																	**92143**
40/12-13	116569																	**116569**
41/12-13			30532															**30532**
55/12-13			23682															**23682**
63/12-13	99102																	**99102**
68/12-13	99102																	**99102**
73/12-13	2000																	**2000**
76/12-13			25682															**25682**
86/12-13	99102																	**99102**
93/12-13			23682															**23682**
98/12-13					150000													**150000**
102/12-13	99102																	**99102**
110/12-13			23682															**23682**
123/12-13	99102																	**99102**
124/12-13			23682															**23682**
134/12-13	99102																	**99102**
140/12-13			23682															**23682**
148/12-13	128453																	**128453**
153/12-13	197409																	**197409**
156/12-13																862		**862**
159/12-13						6930												**6930**
160/12-13				14760														**14760**
161/12-13				13500														**13500**
162/12-13				16620														**16620**
164/12-13				8100														**8100**
166/12-13			23682															**23682**
167/12-13	103295																	**103295**
169/12-13								1356										**1356**
172/12-13						709												**709**
176/12-13									7781									**7781**
177/12-13							1720											**1720**
181/12-13																931		**931**
185/12-13						5000												**5000**
188/12-13								446										**446**
188a/12-13													3950					**3950**
190/12-13				12875														**12875**
191/12-13				12610														**12610**
192/12-13				14735														**14735**
194/12-13				7920														**7920**
195/12-13			34082															**34082**
196/12-13						5034												**5034**
197/12-13						5000												**5000**
198/12-13									7238									**7238**
199/12-13	103295																	**103295**
202/12-13														3900				**3900**
203/12-13						3698												**3698**
204/12-13																781		**781**
205/12-13				6110														**6110**
206/12-13				11020														**11020**
207/12-13						0												**0**
Total	**1522062**	**0**	**322364**	**118250**	**150000**	**26371**	**1720**	**1802**	**15019**	**0**	**0**	**0**	**3950**	**3900**	**0**	**2574**	**0**	**2168012**

BR No	110	120	130	142	152	210	222	223	236	237	300	330	410	418	420	821	840	Total
321-31-3370 Research on Pineapple																		
3/12-13									5125									**5125**
4/12-13						5000												**5000**
8/12-13				3000														**3000**
10/12-13				10460														**10460**
11/12-13				9970														**9970**
16/12-13									7090									**7090**
17/12-13																734		**734**
18/12-13							3215											**3215**
24/12-13																700		**700**
27/12-13				14135														**14135**
29/12-13				11470														**11470**
33/12-13									4964									**4964**
34/12-13												1440						**1440**
35/12-13							1704											**1704**
39/12-13																673		**673**
43/12-13				7800														**7800**
45/12-13				14700														**14700**
46/12-13				14370														**14370**
48/12-13														1500				**1500**
49/12-13						0												**0**
50/12-13									6279									**6279**
51/12-13						5000												**5000**
52/12-13						16413												**16413**
53/12-13																716		**716**
54/12-13										500								**500**
57/12-13				7650														**7650**
58/12-13				3105														**3105**
59/12-13				13650														**13650**
61/12-13				11580														**11580**
62/12-13				12495														**12495**
64/12-13									7287									**7287**
65/12-13							25											**25**
66/12-13						569												**569**
69/12-13																673		**673**
70/12-13				12360														**12360**
71/12-13				13645														**13645**
72/12-13				6000														**6000**
74/12-13				10735														**10735**
79/12-13				5530														**5530**
80/12-13						3498												**3498**
81/12-13						2008												**2008**
82/12-13									8776									**8776**
83/12-13																673		**673**
88/12-13				7050														**7050**
90/12-13				8940														**8940**
91/12-13				12995														**12995**
96/12-13									9639									**9639**
99/12-13						7957												7957
100/12-13																673		**673**
104/12-13				7650														**7650**
107/12-13				14935														**14935**
114/12-13									7923									**7923**
115/12-13																689		**689**
120/12-13				14315														**14315**
121/12-13				12440														**12440**
122/12-13				6900														**6900**
125/12-13									7872									**7872**
127/12-13						3525												**3525**
129/12-13						8467												**8467**
130/12-13						8467												**8467**
132/12-13												1792						**1792**
133/12-13																1010		**1010**
136/12-13						1064												**1064**
137/12-13				9120														**9120**
142/12-13				7500														**7500**
144/12-13				12765														**12765**
147/12-13														1488				**1488**
150/12-13														1400				**1400**
151/12-13													2050					**2050**
152/12-13									8707									**8707**
Total	**0**		**0**	**297265**	**0**	**61968**	**4944**	**0**	**73662**	**500**	**0**	**3232**	**2050**	**4388**	**0**	**6541**		**454550**

BR No	110	120	130	142	152	210	222	223	236	237	300	330	410	418	420	821	840	Total
321-31-3500 Research on Passion fruit																		
12/12-13				10010														**10010**
28/12-13				12925														**12925**
30/12-13				11575														**11575**
36/12-13						0												**0**
37/12-13						5000												**5000**
44/12-13				14755														**14755**
60/12-13				10760														**10760**
89/12-13				4470														**4470**
92/12-13				13760														**13760**
94/12-13						0												**0**
95/12-13						5000												**5000**
105/12-13				12555														**12555**
106/12-13				3220														**3220**
119/12-13				13125														**13125**
143/12-13				5750														**5750**
154/12-13						1820												**1820**
																		124725
Total	**0**	**0**	**0**	**112905**	**0**	**11820**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**124725**
321-31-9027 KSCSTE-SRS Project 'Evaluation of passion fruit types for commercial cultivation in Kerala'																		
6/12-13						5000												**5000**
9/12-13				9220														**9220**
13/12-13		6667																**6667**
15/12-13				10000														**10000**
19/12-13											4004							**4004**
20/12-13												5000						**5000**
22/12-13						29439												**29439**
26/12-13		7903																**7903**
42/12-13		10000																**10000**
47/12-13				8795														**8795**
56/12-13		10000																**10000**
75/12-13		12000																**12000**
85/12-13															166959			**166959**
87/12-13		10000																**10000**
101/12-13						0												**0**
103/12-13		8065																**8065**
108/12-13				4600														**4600**
109/12-13				14145														**14145**
111/12-13												0						**0**
116/12-13											5000							**5000**
117/12-13		10000																**10000**
118/12-13				12650														**12650**
128/12-13															7695			**7695**
131/12-13															7990			**7990**
138/12-13						5000												**5000**
141/12-13		7742																**7742**
145/12-13				14565														**14565**
146/12-13				10580														**10580**
155/12-13											5010							**5010**
157/12-13						3283												**3283**
163/12-13				9360														**9360**
165/12-13		9034																**9034**
170/12-13															24812			**24812**
171/12-13															6872			**6872**
173/12-13															32088			**32088**
174/12-13											0							**0**
175/12-13						0												**0**
178/12-13						6000												**6000**
179/12-13															5080			**5080**
180/12-13																	420	**420**
183/12-13															18504			**18504**
184/12-13				85													580	**665**
186/12-13						1278												**1278**
189/12-13		8835																**8835**
193/12-13		644																**644**
Total	**0**	**100890**	**0**	**94000**	**0**	**50000**	**0**	**0**	**0**	**0**	**14014**	**5000**	**0**	**0**	**270000**	**0**	**1000**	**534904**
Stn Total	**1522062**	**100890**	**322364**	**622420**	**150000**	**150159**	**6664**	**1802**	**88681**	**500**	**14014**	**8232**	**6000**	**8288**	**270000**	**9115**	**1000**	**3282191**