BIOTECHNOLOGY IN AGRICULTURAL DEVELOPMENT

Department of Agriculture
Diversified Agricultural Research Project
Sri Lanka

USAID

Cover Photo : *In vitro* plantlets of pineapple

BIOTECHNOLOGY
IN
AGRICULTURAL DEVELOPMENT

PROCEEDINGS OF THE
BIOTECHNOLOGY WORKSHOP
12 JUNE 1992
BMICH
COLOMBO

Editors

P. GANASHAN and **S. BALENDIRA**
Plant Genetic Resources Centre
Peradeniya

1993
DEPARTMENT OF AGRICULTURE
USAID DIVERSIFIED AGRICULTURAL RESEARCH PROJECT
PERADENIYA, SRI LANKA.

Citation: Ganashan, P., and S. Balendira, Eds. 1993. Biotechnology in Agricultural Development : Proceedings of the Biotechnology Workshop, 12 June 1992, Department of Agriculture, Sri Lanka.

Published by

Director of Agriculture,
Department of Agriculture,
P.O. Box 1, Old Galaha Road,
Peradeniya,
Sri Lanka.

Copies available at

Plant Genetic Resources Centre,
P.O. Box 59,
Peradeniya,
Sri Lanka.

Printed at:

Middleway Limited
257, Union Place,
Colombo 2,
Sri Lanka.

Financial support provided by the United States Agency for International Development (USAID) through the Diversified Agricultural Research Project (DARP) in Sri Lanka.

Contents

POSTERS

Foreword

The agricultural policy directions being pursued by the Ministry of Agricultural Development and Research rely on the twin strategies of promoting crop diversification and on catalysing a market orientation to agricultural sector in Sri Lanka. The Department of Agriculture (DOA) in responding to this strategic policy shifts of the Ministry, has directed its attention to the development and dissemination of technologies to meet the emerging needs of the domestic and export markets.

To illustrate the point let me take an example of immediate relevance. There is a big demand for disease free high quality planting material of elite varieties of fruits such as pineapple, banana etc. to service the market demand for these fruits. Conventional technologies alone are inadequate to meet these newly emerging requirements and DOA is continuously called upon to provide the technology to meet these challenges. There are numerous other needs, such as overcoming the present yield stagnation experienced in our principal crop rice, which need viable solutions. Many developing countries, such as India have in the recent past profitably exploited advances in biotechnological research in several spheres of agriculture. In Sri Lanka too, several government, semi-government and private sector institutions are utilizing such advances in diverse agriculture programmes. But the degree of awareness, identification of end user needs, information on ongoing research and sharing of experiences in such research appears to be limited.

It is the recognition of these facts that led to the organising of a one day workshop on "Application of Biotechnology" under the chairmanship of the Hon. Minister of Agricultural Development and Research. I am extremely pleased that the DOA provided a suitable forum through this workshop for the Sri Lankan scientists, policy planners, administrators, private sector entrepreneurs and media personnel to come together to share experiences, assess current status, identify priorities, set joint targets and discuss management concerns.

The workshop was well planned and was a timely activity with over 100 concerned participants joining together in the sessions. As a policy planner, I am particularly pleased to have participated in all sessions and it was a good brain storming experience to me. I am confident that the recommendations of the workshop will certainly help us in formulating our national policy on the application of biotechnology in agriculture. I must congratulate Dr. S.P.R. Weerasinghe, Director of Agriculture and his editors for bringing out the proceedings in the present form and for maintaining high standards of publication. USAID/DARP is gratefully acknowledged for the generous support given for sponsoring workshop and for bringing out this valuable publication.

Dixon Nilaweera
Secretary
Ministry of Agricultural
Development and Research

Preface

In the recent past, plant biotechnology activities have gained momentum at national level in Sri Lanka. Several government, semi-government, university and private sector institutions are now involved in agriculture related biotechnology research and development activities. Clearly, for a developing and a largely agriculture based country like Sri Lanka, it is prudent to keep abreast with the rapid developments in this field. But biotechnology research is expensive, requires trained personnel and comprehensive knowledge access systems. Thus in the national context, and to optimise on the limited quantum of resources that can be set apart, it is necessary to link the different national programmes, encourage co-ordinated efforts and avoid wasteful duplication. Involvement of the private sector entrepreneurs and awareness among the policy makers and political leadership are equally important in this regard.

It was thus appropriate for the Department of Agriculture (DOA), as the nodal organisation concerned with agricultural development in the country to take the lead in organising a one day workshop on agricultural biotechnology. The workshop provided the much needed forum for the political leaders, policy makers, concerned agricultural scientists, university academics, private sector entrepreneurs, donors and media personnel to come together and:

a. review the current status and examine the pattern of agricultural biotechnology research and development in the country,

b. assess future needs, set research and development goals, develop collaborative mechanisms and analyse some of the issues and concerns,

c. create an awareness among the political leadership, policy makers and the public on the technological possibilities,

d. formulate a National Policy on Biotechnology.

Participants from the different institutions found the ideas that emerged from this workshop to be relevant and very useful in their respective programme development. The publication of the proceedings is intended to bring to a wider audience the consensus reached at the workshop and stimulate further the already vigorous programmes in plant biotechnology that are developing in the country.

The proceedings start off by focussing on the perspectives. Following an overview on the role of biotechnology in national agriculture, the inaugural address looks into the social and economic implications of biotechnology in agriculture. The perspective on crop improvement serves to set the agenda of the workshop. A selected collection of technical papers under the twin themes of "Generation of Technology" and "Application of Technology" and poster papers are presented. Recommendations of the workshop

are included with a view to draw special attention of all those concerned with plant biotechnology. The conferees were able to focus sharply on the research and development needs and develop collaborative mechanisms for future work in this field. I hope that the proceedings will be of specific interest to all the readers.

Let me take this opportunity to recognise Hon. R. M. Dharmadasa Banda, Minister of Agricultural Development and Research for his vision in initiating this workshop and Mr. Dixon Nilaweera, Secretary, Ministry of Agricultural Development and Research for his encouragement and for writing the foreword. I thank Dr. S. L. Amarasiri Deputy Director (Research), other members of the Directorate for their suggestions and co-operation. Special word of thanks to Mr. S. Wirasinghe, Deputy Director (Technology Transfer), Messers K.N. Mankotte, P.W. Weerakkody, R.R.A. Wijekoon, P. Vasudevah and their staff for their dedicated support. The help received from Ms. S. Coorey and Ms. S. Piyadasa in typing the manuscripts is acknowledged. Grateful thanks also goes to Drs. Preston Pattie, S.T.W. Kirinde and USAID/DARP who generously came forward with their support to make this workshop and the publication a reality. Appreciation is extended to all contributors for their timely co-operation and to the invitees for their welcome participation in the workshop. Dr. P. Ganashan and Mr. S. Balendira are specially recognized for their excellence in editing the workshop papers into shape for these proceedings and for co-ordinating the publication.

Dr. S.P.R. Weerasinghe
Director of Agriculture

Sponsor's remarks

Since 1985 the Diversified Agriculture Research Project (DARP) has helped strengthen the Department of Agriculture to carry out its research, extension, and other programmes in support of the agricultural sector of Sri Lanka. The Project is funded by the United States Agency for International Development (USAID).

One of the important contributions of DARP has been to help Sri Lankan scientists establish and maintain professional contacts with others working in related technical fields. This has been done through a massive participant training programme, study tours, attendance at international workshops, obtaining scientific literature, and also sponsoring local workshops, seminars, and conferences.

It was with pleasure that USAID was able to assist the Department of Agriculture in the organization and funding of the present workshop on "Biotechnology in Agricultural Development." Use of tissue culture for rapid plant propagation and the various techniques used in research to improve plants through genetic engineering are becoming widespread. Indeed there are several institutions—public and private—involved in both research and application of this new technology in Sri Lanka, as evidenced by the wide variety of papers and posters presented at this Workshop.

Some of the most important conclusions reached by the participants relate to the need of scientists and practitioners to coordinate efforts among the various institutions involved in this field. This Workshop is an important contribution toward that end. We congratulate the Honourable Minister of Agricultural Development and Research, Mr. R.M. Dharmadasa Banda, for having the vision to initiate this activity, and the Department of Agriculture for providing a forum which brought together nearly 100 persons to participate in this Workshop. It is hoped that USAID will be able to assist with further activities of this kind in the future.

Preston S. Pattie
Chief of Party
USAID/DARP

PERSPECTIVES

Application of biotechnology in agriculture - An overview

Dr. S.P.R. Weerasinghe
Director of Agriculture

Hon. Minister of Agriculture Development and Research, State Minister, Secretary, distinguished guests, ladies and gentlemen.

It is my great pleasure to extend a cordial welcome to this workshop on "Application of Biotechnology in Agricultural Development". About two months ago the Hon. Minister of Agricultural Development and Research suggested that a biotechnology workshop be organized by the Department of Agriculture (DOA). I was somewhat hesitant at first, because Biotechnology, *per se*, has wide applications in agriculture (plants and animals) and in human health. A workshop pervading such complexity and dimensions was not only impractical but would not be meaningful for DOA to take the initiative. The logical course was to scale down and limit the deliberations to plants with a greater focus on the food crop sector.

Upon reflection, however, it dawned on me that it is by a strange quirk of fate that I was given the responsibility of initiating a Biotechnology Workshop. I say this because, 25 years ago, in 1967 I enrolled as a graduate student and completed my post-graduate studies in the Laboratory of Cell Physiology of Professor F.C. Steward, who was then an acknowledged authority on cell physiology and plant tissue culture.. On my return to the country in 1971, I proposed to the DOA on the need for such biotechnology research and twenty years after, it is no doubt a reason for content, that I have been given the responsibility to organize this biotechnology workshop.

Very simply, Biotechnology is defined as "Any technique that uses living organisms or parts of organisms to make or modify products to improve plant or animals or to develop micro organisms for specific uses". Biotechnology is, therefore, primarily a new and refined tool of research. It implies many revolutionary possibilities, in natural science research and development, that has far reaching biological, commercial, socio-economic and legal consequences. Use of biotechnology, is nothing new. For millennia, the technique of biotechnology was applied for brewing beer and wine, making cheese, baking bread etc.

What is different, is that modern biotechnology applies the advances made in cell and molecular biology using two different techniques, i.e. tissue culture and recombinant DNA (r-DNA), popularly referred to as "Genetic Engineering".

Tissue culture is basically a technique where an isolated tissue or individual cells can be grown into whole plants. For example, a cubic centimeter of tissue may contain a million or more genetically identical cells each of which has the capacity to produce a plant. Recent advances in research have opened new vistas in the utilisation of this technique in agriculture. These can be summarised as follows:

- Micropropagation of plant genotypes.
- Induction of genetic variability.
- Elimination of viruses from plant tissues.
- *In vitro* screening and plant regeneration
- Production of haploids.
- *In vitro* Conservation.

Tissue culture has been successfuly utilised with annuals and even with perennial crops. It is now well documented that the time for development of a high yielding oil palm variety has been reduced by a factor of 30, which under traditional breeding methods would have required a scientist to devote his life-time to see results. This technique is, therefore, a valuable tool for plant breeders to speed up the breeding and multiplication of promising genotypes when conventional breeding methods may take a decade or more.

On the contrary, genetic engineering is an intricate technique that isolates a desired genetic characteristic from a plant cell and incorporates into another to produce a variety which has the capacity to perform according to expectations. Its application in crop improvement, increasing efficiency of nutrient utilization and production of useful plant products in agricultural development are rapidly expanding. Noteworthy among them are:

- Transfer of desired genes to established varieties.
- Overcoming of reproductive barriers in non-compatible genera and species for transfer of specific genes.
- Incorporation of Nitrogen-fixing ability among non-legumes.
- Introduction of herbicidal resistance to commercial cultivars.
- Production of plant products like growth hormones.

Plant breeders could thus achieve the desired results in crop improvement by overcoming the natural biological barriers that limit the transference of desired genetic traits between and within species.

Modern biotechnology, therefore, opens new frontiers in agricultural development. And this workshop is being held at an opportune time when agriculture in this country is at cross-roads. There is increasing evidence to show that the food crop sector is reaching production plateaus, amidst rapid population growth and a degradation of the environment consequent to the exploitation of the resource base and excessive use of agro-chemicals.

Recent government initiatives on a market oriented agriculture, inevitably presents new challenges to agriculture development which necessitate a reorientation of the strategies adopted and a search for technologies that could meet the domestic and export market needs. Biotechnology offers much promise to respond to these challenges and it is the responsibility of our national programmes to exploit its potentialities. This is no easy task, for there are wide-ranging problems and implications.

What we see today are several 'biotech' laboratories established in state and private sector institutions. Infact, they have been successful in developing techniques for rapid plant propagation of some plant species and producing disease free planting material in limited quantities. There is, however, a fundamental question that must be addressed by the participants at this workshop. Is it our vision to pursue biotechnology research to meet such limited objectives or should the scope be widened? Such a determination must be necessarily guided by and take cognisance of the fact that success stories of biotechnology research in developed countries had entailed large investmments in infrastructure and man-power.

It is my contention, that Sri Lanka may not be in a position to make investments of such magnitude. Nevertheless, by carefully taking an inventory of and pooling the available physical and human resources, the full potentialities of biotechnology can be exploited for rapid agricultural development.

In doing so, there is a fundamental need to establish mechanisms by which biotechnology research in different laboratories can complement each other thereby preventing duplication. Do we have the determination and can we make a commitment to achieve such aspirations? This would necessarily imply a close co-ordination among biotechnologists and, more importantly, establishing links and collaborating with International Centres of excellence associated with biotechnology research.

Another crucial issue that will surface in such biotechnology research will be the methodology of transferring mature technologies. Particularly, in an era where we are moving towards commercialization of agriculture, the question of patents will be of utmost importance. It will be prudent to examine this issue by a representative team of scientists who could be associated in determining the modalities of co-ordinating biotechnology research in Sri Lanka. Such a team should also examine the implications and prevent the exploitation of the country's genetic resources and tag on patent rights that may prevent the farmers from gaining easy access to the technological advances that are made. In summary, therefore, it is important to recognise that biotechnology has wide applications and can make a profound impact on agricultural development. Even so, it is also imperative to take cognisance of the available resources, determine priorities and the socio-economic implications that may influence its application by small holder resource poor farmers. In such a scenario, it is now opportune for a national commitment in biotechnology to facilitate an accelerated thrust.

In conclusion, it is my fervent hope that the Hon.Minister of Agricultural Development and Research will take the lead in formulating and establishing a National Policy on Biotechnology. *Thank you.*

Inaugural address

Hon. R. M. Dharmadasa Banda
Minister of Agricultural
Development and Research

Distinguished guests and participants

At the outset, I must say how happy I am to be here today, at the inaugural session of the workshop on the "Application of biotechnology in agricultural development in Sri Lanka." I am also keen to participate for another reason. I am involved in farming and represent the farmers from a backward area like Moneragala where agriculture is our mainstay and despite the advances made in agricultural technology there is evidence of only a moderate impact and farmers still exist at subsistence level.

I can hardly open a popular magazine or newsprint these days without finding exciting articles on the potentials of biotechnology. A technology which is proclaimed to contribute towards the alleviation of malnutrition and hunger. These articles have raised my expectations and hope on applying biotechnology for agricultural development. My curiosity and interest in biotechnology increased last February, when I had an opportunity to visit some of the research stations and laboratories in India. India is trying its very best to develop the agricultural sector and has been successful in increasing their agricultural productivity. This has been made possible largely through the valuable contributions made by Indian scientists in introducing new techniques such as tissue culture.

My hopes are based on the potential applications of biotechnology in agriculture in a country like Sri Lanka, for the socio economic upliftment of the vast majority of our rural farmers. I am indeed amazed to read about the bio-engineered, 'blue roses', 'luminous tobacco', etc. I am sure that our scientists are not spending their time on such fancy research or trying to create a "cabbage with carrot roots". But my curiosity is always aroused when I hear about the potential yield increases possible through biotechnology. I recognise the relevance of "super" plants that have been developed to produce their own pesticides thus reducing the need for costly and harmful agrochemicals. About plants that could be grown in poor soils to withstand salinity, drought, etc., about micro organisms that are engineered to attack pests that damage crops. The list seems endless and promises great advantages for agriculture especially in countries such as ours. We are all excited about the coming bio-revolution or gene-revolution in agriculture.

During this workshop, I wish to raise some key issues, in terms of the social, economic and ethical implications. I hope my observations will be considered in formulating policies on the application of biotechnology in agriculture. The Director of Agriculture in his address raised the issue of a policy statement on biotechnology which I appreciate as important.

It is my conviction, that our main focus should be on rice, our staple food. For over two and a half decades we are trying to achieve self sufficiency in rice. Every effort was made to open up new lands to increase paddy production by constructing large and small tanks and reservoirs. In addition, better yielding varieties were introduced to the farmers. With all these endeavours we have not been able to achieve self-sufficiency and still continue to import rice. The quantum of rice imports for 1992 has not been determined but so far we have permitted the importation of 250,000 tons of rice. The statistics received so far show that the yala crop has not given the anticipated production of 40 million bushels. We cannot keep our nation starving, the shortfall will be determined in due course and the country's requirements will have to be imported.

I request our scientists to take a serious note of this situation. Some say, we can become self sufficient by increasing the rice extent by another 50,000 acres. My estimation is that even after increasing the extent by another 50,000 acres, the rice production increases will not be able to feed the additional hundreds of thousands of mouths that will increase every year. We and particularly the scientists must take serious note of these facts. I was told by our scientists that it takes about 6-8 years to produce a new rice variety by hybridization. I presume, now is the time for our scientists to explore all the possibilities of reducing this long period of 6-8 years taken to produce new varieties.

I am certain that you would be successful in that attempt. Then there is the important issue of minimizing post harvest losses. The post harvest losses, as far as rice is concerned, has been estimated at 14 percent. If we can lower it to zero percent - I do not know whether this is possible - then we could reach self sufficiency without any effort. Some may argue that it would be a miracle to reduce post harvest losses to zero percent but can't we reduce this to a single digit in a targeted period? By increasing the acreage, introducing new high yielding varieties and minimizing post harvest losses, I think we can reach self sufficiency within a limited period.

We produce quality paddy like any other Asian country. But the rice that is marketed in the country is not of the high quality as that in other Asian countries. Poor milling standards appear to have downgraded the quality that is inherent in our varieties. Efforts to rectify this situation do not require high level of technology. I have instructed institutes like the Paddy Marketing Board (PMB) and private sector millers to produce quality rice and I have seen positive reslts without any high technology. I urge this worksop to take post harvest processing also into consideration. With vegetables, post harvest losses have been estimated to be as high as 40 percent. Is it not our duty to introduce methods to reduce this loss? I hope and wish that our scientists would play their role in due course by introducing new methods to reduce the post harvest losses in vegetables.

All these which I say as a layman to scientists, who have reached international standards, may not be new problems. But I think it is my obligation to share with you my concerns. As special attention must be given to fruits, it is the bounden duty of the scientists to engage in research activities on all species of fruits which can be grown in Sri Lanka. Priority has been given to a few species like papaya, pineapple, banana, mangoes and very recently rambutan. This does not mean that other fruits have to be neglected.

Now take the case of papaya, it can be grown anywhere in our country and the taste is comparable to that found in other Asian countries. What about the demand? Some say there is an unlimited export potential for papayas to Japan. They require a specific size, colour and taste. I request the research division to identify the varieties which have the required size and colour. I have already seen about seven varieties which have the required size, colour and taste suitable for the Japanese market. The demand for necessary planting materials of these varieites can be met through applying biotechnology. And it need not be a massive project. With a small scale production programme, in an area within a radius of about 30-50 miles from the airport where facilities for packing and transport are available, this target could be achieved very easily.

Now everybody is talking about pineapple but only a very few know about the variety Kew. This variety is available in our country. It is indeed amazing to note that we have the best soil type in our country to grow pineapples. In other Asian countries where pineapple is grown, the maximum sugar content is only about 12 per cent, while in Sri Lanka it ranges from 17 - 19 per cent. This was tested at CISIR and later confirmed in USA. Our soils can produce the best pineapple in Asia and a ready market is available.

It is high time that our scientists give priority to this crop because we have already started the first nursery of 10 acres and the second nursery of 50 acres is being established. After completing the second nursery, the last nursery of 300 acres is to be started. Once it is done, the planting materials will be distributed to farmers. We are starting a massive project and we may face difficulties initially, but I want the scientists to see that this project gets on well.

We need biotechnology now. Earlier the requirement was 3000 - 4000 plants per acre, but now the per acre requirement is about 25,000 plants. This is one of the examples of the development of technology. We can increase production by using this method. We are planning to establish a 10,000 acre plantation under pineapple only for export. It can be more for domestic consumption. So you can realise the magnitude of the undertaking, when the requirement of planting material is 25,000 x 10,000 and we do not intend to import any pineapple planting materials.

We have to introduce a system of rapid multiplication. This is one area where you will have to play an important role. I am told that a 300 acre nursery will provide planting material only for 1500 acres. When I consulted the Director of Agriculture, I was told that a simple rapid multiplication technique has been developed to produce 15 - 20 suckers from a single plant. What is required is to decapitate the tip of the plants. No sooner I learnt of this technique, I transferred it to the farmers.

Very recently some of our scientists have told me that we could obtain over 6000 plantlets from one plant using a new technology. I want you to advise me on the possibilities of this technology. If such a multiplication programme can be introduced it can benefit the country. Why I say this is because according to the present rate of planting material production, the 10,000 acre plantation can be established only by 1997. And we cannot wait till 1997. I want maximum assistance from our scientists.

Banana is another important fruit crop. Our scientists have disclosed, in their papers, that with the present technology 400-500 plantlets can be produced from one shoot tip. The market prices of bananas are very high in Sri Lanka. I am very happy that the farmers are getting a high price but, on the other hand, consumers are complaining that the prices are too high. A team from Middle East recently met me and assured a market in the Middle East for about 125,000 tons of banana annually from Sri Lanka. We have to produce according to their taste, colour and size which we can do easily. If we can increase the production of suitable banana planting materials by applying biotechnology, not only we can produce to meet the domestic demands, but also for the export market and earn foreign exchange.

Besides fruits, let us take the case of potato which the Director of Agriculture mentioned. As you may be aware, we did not import into the country consumption potatoes for the past last 18 - 20 years. Some argue that we have not produced enough in this country and they base their arguments on the higher market prices. It is not a secret that we are importing seed potatoes to this country. The first information I got after assuming this office was about the nematode problem in potato. I went to Nuwara Eliya with the concerned officials and certain decisions have been taken which are being implemented. As you all know, the farmers have to plant one ton of seed potatoes to produce 10-15 or sometimes 20 tons of potatoes. Seed potato prices have been rising rapidly. Earlier one ton of seed potato was sold at Rs. 28,000/= but I understand that prices may even rise to about Rs. 80,000/= per ton. How can this be controlled? I wish to state categorically that the prices of seed potatoes cannot be allowed to escalate to Rs. 80,000 - 100,000/= per ton.

Let us see this problem. As a scientist, the Director of Agriculture said we can make use of biotechnology. But still the fact remains that we need annually 4000 tons of seed potatoes, may be farmers are increasing the acreage. When I visited Bandarawela two years ago I was very happy to be told that by introducing the tissue culture technique, the entire requirement of the Badulla district could be met within two years, if green houses and other facilities are provided. If there is a possibility as stated by the Director,

why are we spending millions of dollars to import seed potatoes. These methods do not cost so much. If we can develop suitable techniques, then I think we need not import seed potatoes or give government farms to the private companies to produce seed potatoes. I hope you will start this programme within the shortest possible time.

The excitement regarding the possibilities of this new bio-revolution reminds me of the mood when the results of the earlier green revolution started to reach our farmers fields. During the earlier years of this revolution, substantial increases in the production of rice and other cereals were achieved. Now, nearly quarter century later and after thousands of reports on the effects of green revolution, let us pause to consider the benefits and concerns of the first revolution.

Certainly we all agree green revolution technology was a package of yield enhancing technology. In Sri Lanka there was a quantum jump in terms of national average yield of rice from 1.5 tons/ha in 1949 to 3.6 tons/ha in 1988. This was more than double. That technology was scale neutral and farmers reaped the benefits irrespective of their holding sizes. It paved the way for rural development, renewed self confidence in farmers and created avenues for upward social mobility in rural areas. In real terms it also stabilized our food prices. Sri Lankans were not forced to eat cassava or sweet potato, thanks to the bountiful harvest of rice. Above all, the green revolution encouraged successive governments to give political priority to agriculture. But green revolution technology also had in-built limitations. The yield output was related to costly inputs. This is where the green revolution by-passed the very resource-poor rural farmer.

Can this new bio-revolution help us to produce crops which can respond to limited fertilizer inputs? Can we produce crops which can withstand pests and diseases? How can we reduce the cost of production without reducing the yield? This is where the new interest in biotechnological approaches rests. Our aim is to enhance the stability and sustainability of the production process. Obviously there is a need to apply biotechnology in close integration with the ongoing crop improvement research.

The programme of this workshop clearly points out the directions in which Sri Lankan scientists are embarking upon. They are trying to find solutions to well defined problems and are not trying to worship at the altar of specific disciplines or tools of research. There is a suitable blend of conventional and newly emerging biotechnology which I am sure will add new dimensions to our agricultural research capabilities. And I am hopeful that this technology will realy serve the needs of the poorer farmers of this country.

Director of Agriculture posed me two questions. One is a request to introduce a policy note on biotechnology, which I am prepared to do. Then he wanted certain assistance to develop this sector. He himself has confessed that we are a poor nation and we cannot set apart billions of dollars for this sector. It is true, but we will do our best. I am hopeful that if you can produce results, we are in a position to give you as much assistance as you require.

I would like in conclusion to point out that, somewhere in 1968, parallel with the commencement of the green revolution, American scientists were very busy conducting space experiments. President Kennedy was determined to win the space war and had confidence in the capacity of their scientists to do so. He set a deadline to reach the moon before the Russians. That was the challenge for the scientists in America. He gave all the assistance. The scientists did their best and won the war by sending the Americans to the moon on schedule. That is how battles and wars are won. We are also at war. At the start, I said we have to be self sufficient in rice. Not only in rice but in all crops.

I thank the Director of Agriculture for organising this workshop because this is an opportune time to discuss biotechnology. I also thank his staff for all the assistance given to make this workshop a success and to all of you for attending. I hope you will have fruitful discussions, and at the end make positive recommendations. *Thank you.*

Biotechnology in crop improvement

P. Ganashan
Head
Plant Genetic Resources Centre

Introduction

Biotechnology, considered in its broad sense as the manipulation of biological systems, is described as the latest technological advancement of the 20th century. Although, the practical applications of biotechnology, such as the use of yeast cultures to make curd or fermenting toddy have been with us for centuries, the breakthroughs made since 1970's have great potentials in the fields of agriculture, health and industry.

The first leap forward in this direction came with the rediscovery of Mendel's principle of heredity in the beginning of the present century. By the 1950's the science of genetics was helping rice breeders in Sri Lanka to release improved rice varieties such as H_4 and H_7 and boost productivity. However, the yields obtained were not comparable to the productivity of the "japonica rices", due to want of genetic potentials among our "indica rice" group. The innovative mutation induction technique was tried to make yield gains. MI 273 (mutant) which resulted from the gamma ray irradiation of H4 rice and named "Dwarf H_4" resulted in further yield increases.

The discovery of the dwarfing gene in the spontaneous mutant "Dee-Geo-Woo-Gen", a Chinese indica rice and its extensive use in rice breeding programmes changed the entire outlook of the production potentials of the Sri Lankan agriculture. The introduction and wide adoption of the resultant semi dwarf, photo-insensitive, high yielding "Bg Series" of rice in the 1970's led Sri Lanka into green revolution.

The green revolution demonstrated that life sciences technology, effectively applied, results in enormous economic and social benefits in any country. With the rapid increases in population, uncertainties of rainfall pattern and diminishing land availability, the emphasis in agricultural crop improvement is moving away from the intensive farming practices on which green revolution was built. Further, the green revolution technology was developed at a time when petroleum derived energy and inputs were cheap. The need now, is for technologies which reduce dependance on costly chemical fertilizers, pesticides and herbicides while sustaining crop productivity. Towards this end, biotechnology provides unique opportunities for accelerating the pace of plant breeding and promises a new gene revolution in agriculture.

Plant biotechnology in agriculture

The foundation for this gene revolution was laid even before the fruits of green revolution started sweeping the agricultural landscapes in Asia. The discovery of the double helix DNA structure by two scientists Francis Crick and James Waston in 1953 transformed the science of genetics and opened a new frontier in genetic manipulations. The chemical DNA carries hereditary information and is capable of replicating itself. In 1971, the technology to rearrange DNA and alter genetic make up of living things, through transferring specific genes from one organism to another, was developed. By the turn of 1980's the limitations in the conventional crop improvement technologies to meet challenges were fully recognised and biotechnological approaches began to be considered.

The fundamental bases of modern biotechnology are the two different techniques that have been developed and improved in the past decade: tissue culture and recombinant DNA (r-DNA) techniques.

Tissue culture techniques enables scientists to grow the early stages of plant in a laboratory environment, *in vitro* (literally "in glass"). "Tissue culture" is a general term covering all forms of *in vitro* culture. The plant can be started from tissue (an aggregate of similar cells) or from a single cell, from an embryo, or from other sources such as the anther (part of the male reproductive organ in plants).

The possibilities resulting from recombinant DNA technologies are even more far reaching. Recombinant DNA enables breeders to isolate desired genetic traits from one cell and incorporate them to another. Micro organisms or parts of them are used as vectors in the transfer of genes. This technique offers the means to carry crop improvement at cellular level and in a way directed towards desired results. The years it takes for genetic improvements through traditional breeding could be reduced to a fraction of that time and genes can be transferred not only within species, but among species.

Effective crop improvement programmes, both conventional and biotechnological, depend on the availability of a reservoir of plant genetic resources which forms the basic building blocks. Biotechnology can enhance directly the economic value of this gene wealth or germplasm and can create new gene combinations for use in breeding programmes. The biotechnological approaches used in direct application on available germplasm are:

- Germplasm collection : *In vitro* collecting techniques are now available for field collection of wide range of vegetatively propagated crops.
- Rapid clonal multiplication: a commercially practical means for propagating plants rapidly, uniformly and free of diseases without climatic and seasonal restrictions.

- *In vitro* conservation: a new technology to conserve a range of vegetatively propagated crops under slow growth and cryopreservation conditions for present and future use.
- Pathogen free plant introduction: tissue culture techniques are very valuable in plant quarantine for introducing disease free crops from other countries.
- Molecular diagnostics: the use of techniques such as Restriction Fragment Length Polymorphism (RFLP), Polymerase Chain Reaction (PCR), Isozymes etc., to assess, identify and tag genes of economically important traits. Antibody and molecular techniques also provides realiable and sensitive procedures for disease indexing.
- DNA Storage: long term storage of DNA provides a viable option for maintaining genes tagged for their usefulness.

The biotechnological tools available to create new gene configurations or to enhance germplasm are:

- Embryo rescue: used to rescue plants resulting from crossing incompatible species and is a widely incorporated technique in breeding work.
- Somaclonal variation: generating new genetic variation for evaluating, selecting and isolating new genotypes. *In vitro* mutagenesis offers possibilities to carry out selection at cellular level for biotic and abiotic stresses.
- Anther culture: incorporated in breeding programs to reduce breeding time.
- Genetic engineering: practical methods for transfer of alien genes which cannot be incorporated through normal crossing methods due to crossability barriers, are now available for application in crop improvement programmes.

Progress of biotechnology in the Department of Agriculture

The role of biotechnology in agriculture in countries such as Sri Lanka is specific. It is a supplemental innovation that conventional crop breeding methods so badly need to develop improved crops. It is in this context, the Department of Agriculture (DOA) sets out to build the base in biotechnology. The Department utilises scientific advances made in advanced research institutions in developing tools for its own work. In adapting biotechnologies, the DOA develops internal capabilities to take advantage of new ideas and tools and identify problems that are appropriate for biotechnological solutions.

Tissue culture techniques were pioneered by DOA in 1976 to support the demand for rapid clonal multiplication of orchids and anthuriums for cut flower industry. In 1984, tissue culture activities were expanded to fulfill the need for pathogen free micropropagation of fruit crops such as pineapple, banana, citrus, passion and papaya.

The assistance of FAO was obtained to establish a tissue culture laboratory at Central Agricultural Research Institute (CARI), Gannoruwa, for this purpose. Subsequently, the program was enlarged to include micropropagation of rambuttan, strawberry, ginger etc. Research on minituber production of potato varieties was undertaken and technology has been perfected.

Micropropagation of potato through apical meristem culture to obtain disease free planting materials, was initiated at Regional Agricultural Research Centre, Bandarawela in 1987. The mass propagated disease free stem cuttings are distributed to farmers for their own seed production programme since 1989. The potato minituber technology is also being used to produce basic seed.

The Plant Genetic Resources Centre (PGRC), Gannoruwa, was established in 1988 with the assistance from Japan International Co-operation Agency (JICA), and has capabilities to undertake plant genetic conservation and conduct biotechnology research. Programmes are underway for conserving genetic resources in the form of seeds, *in vitro* and in the field. Strategic research on callus culture, embryo culture, anther culture, protoplast culture are conducted on a number of crop species and suitable protocols have been established. The aim of these research programmes is to support crop improvement programmes in genetic enhancement aspects. Efficient micropropagation technology for selected fruit crops has been made available. Biochemical techniques such as isozyme analysis are conducted to assess genetic variability and identify economically important traits, for use in crop improvement activities.

Conclusion

There is a great potential for biotechnology in Sri Lanka as a tool in crop improvement programmes. Genetic manipulation of plants involving tissue culture, recombinant DNA, restriction fragment length polymorphisms, isozymes and incorporation of genes from wild species forms the basis of biotechnology. PCR technique helps to detect expression of desired genes in the early segregating populations. The biotechnology programmes of the DOA are closely integrated to meet the specific breeding objectives. Techniques such as anther culture, embryo rescue have been incorporated in the breeding programmes. Biotechnology is used to alter the genetic background of crops to suit the environment, and to meet the needs of the resource poor farmers. Therein lies its promises for economic and ecological sustainability.

SESSION 1

GENERATION OF TECHNOLOGY

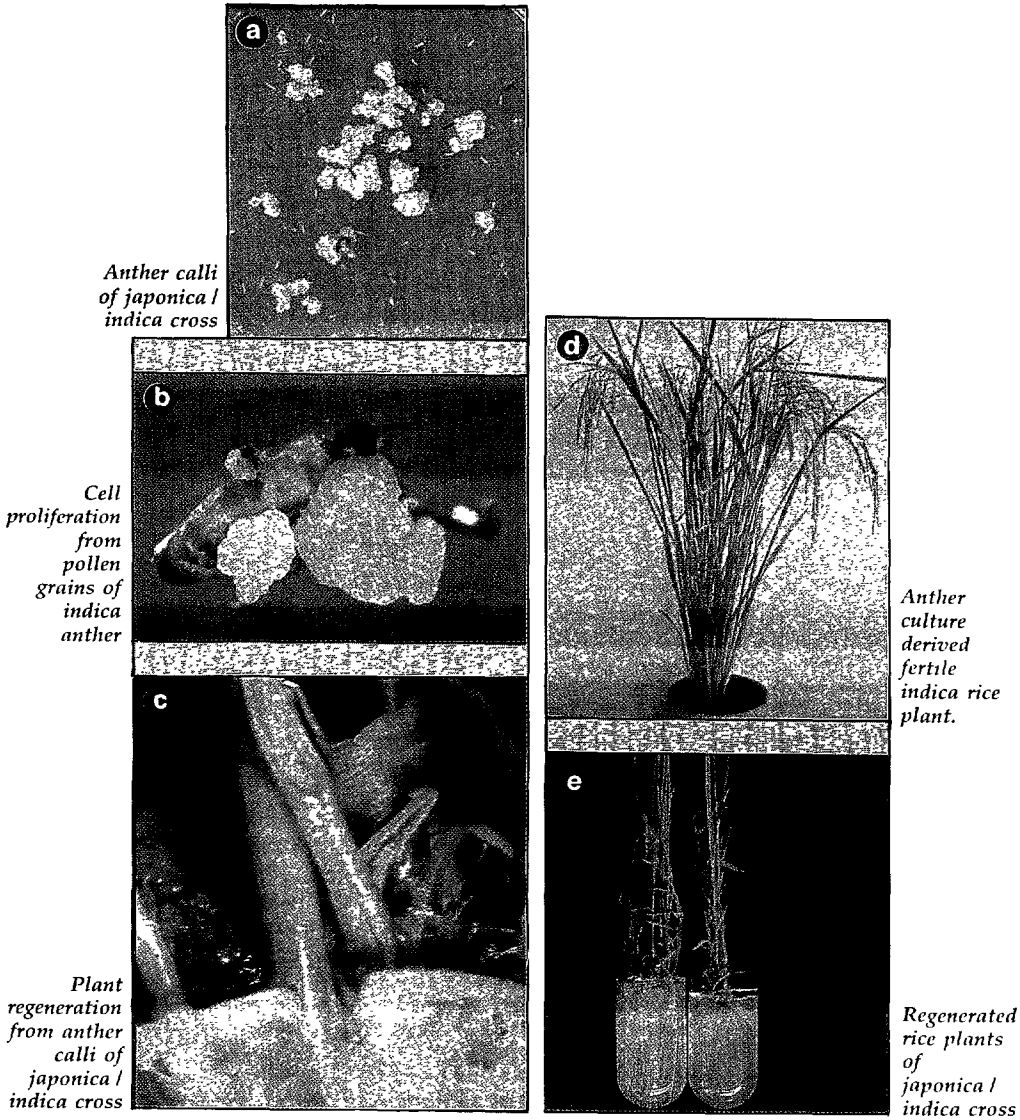


Plate 1. ***Rice anther culture***

Application of innovative techniques for rice breeding

M.H. Mendis, A. Hettiarachchi, M.P. Dhanapala and P. Ganashan

INTRODUCTION

In Sri Lanka, conventional breeding programmes in rice have resulted in the development and release of promising "Bg" and "Bw" series of rice varieties. These varieties have genes predominantly from Cina and Latisal both contributing from 18.8 to 50.0 percent nuclear gene sources (Javier *et al.* 1989). All the semidwarf varieties presently in use have their dwarfing gene from Dee-geo-woo-gen. Their resistance to gall-midge was incorporated from OB 677/678 and for brown plant-hopper from PTB-33. Instances of breakdown in resistance to these insect pests and for blast disease have now been reported. Breeding for abiotic stresses such as salt, drought and adverse soil conditions also faces general difficulties. In this context, plant biotechnology offer possibilities to transfer genes across incompatibility barriers, and also speed up the varietal development process.

Traditionally cultivated indigenous rice varieties have over years proved that they are genetically resistant to pests, diseases and abiotic stresses such as flood, drought, salinity etc. Poor combining abilities of these genetical traits have greatly disappointed the conventional breeders in their efforts to improve rice. Japonica rice originating from high altitude temperate regions could contribute the cold tolerance needed during vegetative period. Transfer of such valuable characters from japonica rice to indica types by conventional breeding is very difficult due to partial sterility of the hybrids and the subsequent segregation of characters in succeeding generations. Conventional crossing of cultivated rice with wild rice species which are sources of useful characters is possible but it is extremely difficult as the embryo usually aborts after 14 days. Presently, in rice, Sri Lanka has reached a point where further increase in productivity may not be easily achieved without a major breakthrough in technology. Rice breeders have realised the potential of tissue culture techniques in breeding high yielding cultivars with better adaptability to environment. Anther culture and embryo rescue techniques are identified as most appropriate at present in order to improve rice cultivars in Sri Lanka. Anther culture has the potential of compressing breeding cycles, increasing selection efficiency, providing for early expression of recessive genes, and exposing gametoclonal variants.

Rice was the first cereal to be regenerated into plants from cultured tissues (Yamada and Loh 1984). The first reports of plant regeneration of rice were from root (Kawata and Ishihara 1968), anther (Niizeki and Oono 1968) and embryo (Maeda 1968 and Tamura 1968) derived callus. Since then several other workers have reported obtaining plants from other explants such as ovary (Zhaou and Yong 1981), leaf sheath (Bhattacharya and Sen 1980), leaf blade (Yan and Zhao 1982), immature panicles (Ling *et al.* 1983)and anthers (Raina *et al.* 1989, Quimio and Zapata 1990).

Jena and Khush (1985) using embryo rescue techniques, presented the possibility of gene transfer from wide hybridization. Successful trait transfers include resistance to brown planthopper and whitebacked planthopper from *O. officinalis* and bacterial blight from *O. longistaminata* (Toenniessen *et al.* 1989).

MATERIALS AND METHODS

Anther culture

Panicles at the reproductive stage were collected from indica rice varieties B 4403 F-MR-17-1, Bg 94-1, Bg 276-5, Pokkali, IR 1552 and F_1 crosses of Hua Lien Yu 173 / Bg 1222, Hua Lien Yu 173 / Bg 850-2 and Hua Lien Yu 173 / Bg 300 (japonica / indica) grown in the green house. They were kept at 8°C for 8 days and were surface sterilized with 30% (v/v) clorox solution (containing 5.25% w/v NaOCl) for 20 minutes and rinsed three times with sterile tap water. Anthers at the medium uninucleate to early binucleate stage were selected and plated in five different media (FJ_1, FJ_2, AH_1, AH_2 and AH_3), agar (0.8 % w/v Wako pure) for callus induction. These culture media were based on N6 medium (Chu *et al.* 1975). The plated anthers were incubated in the dark at 25°C for callus establishment. Following an incubation period of 4 - 8 weeks, calli formed were transferred to five agar - solidified media (SK_{11}, M_1, M_4, M_5 and A_1) which were based on MS (Murashige and Skoog 1962) medium for regeneration of plants. The callus cultures were maintained at 26 ± 1°C under fluorescent light (1000 - 2000 lux, 16h). Green shoots which emerged from the calli were transferred to hormone - free MS medium (agar solidified) for root induction. Chromosome numbers of regenerated plants of all indica rice were observed. Green plants derived from the anther callus were transferred to soil.

Embryo rescue

O. sativa cv Bg 94-1 plants were crossed with *O. nivara*, *O. eichingeri*, and *O. rufipogon*. The immature seeds were collected within 10 -14 days of pollination and surface sterilized using clorox solution (30% v/w) for 20 minutes followed by washing with sterile tap water. Developing embryos of these immature seeds were dissected and transferred to MS medium containing 0.2 mg/l NAA and KN. Cultures were maintained at the same environmental conditions mentioned for anthers. The developed plantlets were transferred to soil.

RESULTS

Anther Culture

Callus formation from cultured anthers was observed within 4 - 6 weeks of culture. The microspore - derived callus was compact and white or light yellow with white patches. Nodules appeared on white patches of the callus before transferring to plant regeneration medium. These nodules increased in size with the increase of incubation period and finally developed into embryoids upon transfer to plant regeneration media. Green or albino shoots developed from these embryoids. The green shoots produced roots when transferred to hormone free medium (Plate 1). A few plants formed from indica calli were found to be haploids. All other plants were diploids. The results obtained from the F_1 anthers of Hua Lien Yu / Bg rice varieties are summarized in Table 1. Some of the regenerated plants exhibited spikelet sterility. Callus formation was not observed in the cross combination Hua Lien Yu / Bg 300 in all the media used.

Table 1. Callus induction and plant regeneration from F_1 anthers of the crosses between Hua Lien Yu and Bg rice varieties.

Materials	Callus induction		Plant regeneration	
	Medium	No. of callus	Medium	No. of plants
Hua Lien Yu/Bg 1222	FJ	25	M_1	08
	FJ_2	20	M_4	10
	AH_1	32	M_5	03
	AH_2	26	SK_{11}	15
	AH_3	10	A_1	24
Hua Lien Yu/Bg 850-2	FJ	20	M_1	03 *
	FJ_2	23	M_4	06 *
	AH_1	29	M_5	09 *
	AH_2	21	SK_{11}	11 *
	AH_3	20	A_1	13 *

* albino plants

Embryo rescue

Plants were sucessfully established from the cultured embryos of cultivated / wild rice crosses. These plants grew well upon transfer to soil. The morphology of these plants was intermediate when compared with those of the parentals. Viable seeds could be obtained from the crosses with *O. nivara*.

DISCUSSION

The major rice breeding objectives include yield and quality improvement, incorporation of genetic resistance to biological stresses such as diseases and insects, tolerance for environmental stresses like low temperature and salinity and the incorporation of desirable traits from closely related species. Developing a rice variety by conventional breeding takes about 8-10 generations to combine and fix desirable characters. Diploids obtained from anther culture not only shorten the time needed to develop new breeding lines for evaluation but also allow the expression of recessive genes in the population established from anthers of F_1 hybrids.

In the present study it was possible to establish callus cultures from anthers of all the indica rice varieties and the frequency of callus formation was less than 20 percent. Calli of some varieties (B 4403 F-MR-17-1, IR 1552, Bg 94-1) produced green plants. Occurrence of albino plants is a ubiquitous phenomenon in anther culture studies. The frequency of occurrence of albino plants has been reported to range from 5 - 90% (Chu chih - ching 1982) and it varies among cultivars. Wang *et al.* (1977) have reported that higher temperatures favour the formation of albino plants from cultured anthers. Results from several laboratories have indicated that the proportion of regenerated albino plants is genetically determined (Oono 1975, Chen and Lin 1976). Subsequently Chaleff and Stolarz (1982) reported that production of green plants from cultured rice anthers depends on the composition of culture medium and plant genotype. Callus formation was not observed with the F_1 anthers of Hua Lien Yu / Bg 300 due to low pollen viability of 5 percent observed.

Another obstacle in anther culture of rice is the production of plants with different ploidy levels. (Chen 1980). In the present study only a few plants derived from anther culture were found to be haploids. Nuclear fusion and endomitosis occurring during the early stage of microspore development can be accounted for the formation of diploids. Sterility is another problem observed with microspore derived rice plants. However plants with low fertility can be subjected to another cycle of anther culture to recover fertile plants.

Many rice varieties or new lines have been produced by anther culture techniques and in China over 100 new varieties have been developed. These include varieties exhibiting early maturity, blast resistance, salinity tolerance and high yield (Xu shi - huan 1980, Zapata *et al.* 1989, Ding xue Ying *et al.* 1981).

The primary goal in wide hybridization is the introgression into breeding lines of a small chromosome segment which contains one or more genes from wild species. The traits successfully transferred from wild species to cultivated species include resistance to brown plant hopper (BPH) and white backed plant hopper (WBPH) from 0. *officinalis* (CC) and bacterial blight from *0. longistaminata* (AA). Hybrids have also been established with the *0. latifolia* (CCDD), *0. nivara* (AA) and *0. rufipogon* (AA) species

(Mujeeb-Kazi and Sitch1989). Crosses involving species with AA genome (*0. nivara, 0. rufipogon*) generally have high seed sets and well formed seeds (Mujeeb Kazi and Sitch 1989). *0. perennis and 0. rufipogon* are potential sources of male sterility. The male sterility gene used in China for hybrid rice seed production is from a wild relative.

In the present study, *0. nivara, 0. rufipogon, 0. eichingeri* were crossed with cultivated rice. The former is resistant to grassy stunt virus and the latter is to BPH, WBPH and gall midge. The other species have the tolerance to stagnant flooding in addition to cytoplasmic male sterility. The objective of the study was to develop methodologies to rescue hybrid embryos. F_1 plants were successfully established but often hybrid sterility was a problem. This is due to irregularities occurring during cell division in the formation of gametes. Doubling the chromosomes with colchicine may improve fertility. Anther culture of F_1 hybrids may be useful in establishing homozygous diploid substitution and addition lines. Back crossing can improve fertility with the elimination of undesirable characters that come from the wild species. When there is little or no meiotic recombination of chromosomes between cultivated and wild rice species, somaclonal variants can be regenerated by tissue culture.

Both anther culture and wide crossing of rice was successful with some limitations. The principal aim of the Department of Agriculture (DOA) research system is to increase productivity, stability and sustainability in rice and other food crops. DOA utilizes all the tools of science to fulfil the needs of the country. During the last few years there is an increasing trend, towards the application of innovative techniques in the national rice improvement programme. DOA has initiated the integration of the tools of biotechnology in the conventional rice breeding programmes to achieve the desired objectives.

References

Bhattacharya, P., and S.K.Sen. 1980.Potentiality of leaf sheath cells for regeneration of rice (*Oryza sativa L.*) plants. *Theor. Appl. Genet.* **58**: 87-90.

Chaleff, R.S., and A. Stolarz. 1982. The development of anther culture as a system for *in vitro* mutant selection. Rice tissue culture planning conference, IRRI, Los Banos Laguna Philippines,pp. 63-74.

Chen Chi-chang.1980.Chromosome number changes in microspore callus of rice during successive subcultures. *Can.J.Genet.Cytol.* **22**: 607 - 614.

Chen, C.C., and M.H. Lin. 1976. Induction of rice plantlets from anther culture. *Bot. Bull. Academia Sinica.* **17**: 18 - 24.

Chu Chih-Ching. 1982. Anther culture of rice and its significance in distant hybridization. Rice tissue culture planning conference. IRRI, Los Banos, Laguna Philippines, pp. 47 - 53.

Chu, C.C., C.C. Wang, C.S. Sun, H. Chen, K.C. Yin, C.Y. Chu, and F.Y. Bi. 1975. Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. *Sci. Sin.* **18:** 659 - 668.

Ding xue - Ying, Song Rui-ling, and You Jing - zhu. 1981. The research on elevation of the rate of success in haploid breeding through anther culture. *In:* Studies on anther culture rice breeding, Shen Jin-hua, *et al.* eds.

Javier, E.L., C.A. Sandanyake, and R.M.T. Rajapakse. 1989. Central Rice Breeding Station: Accomplishments and current strategies. Batalagoda. Sri Lanka. p. 17.

Jena, K.K., and G.S. Khush. 1985. Production of monosomic alien addition lines of *O. sativa* having a single chromosome of *O. officinalis*. *Rice Genetics.* **1:** 199 - 208.

Kawata, S., and A. Ishihara. 1968. Regeneration of rice plants, *O. sativa* L., in the callus derived from the seminal root. *Proc. Jpn. Acad.* **44:** 549 - 553.

Ling D.H., W.Y. Chen, M.F. Chen, and Z.R. Ma. 1983. Somatic embryogenesis and plant regeneration in an interspecific hybrid of *Oryza*. *Plant Cell Reports.* **2:** 169 - 171.

Maeda, E., 1968. subculture and organ formation in the callus derived from rice embryos *in vitro*. *Proc. Sci. Soc. Jpn.* **34:** 139 - 147.

Mujeeb-Kazi, A., and L. A. Sitch, eds. 1989. Review of advances in Plant Biotechnology, 1985-88: 2nd International Symposium on Genetic Manipulation in Crops. Mexico, D.F.. Mexico and Manila, Philippines : CIMMYT and IRRI.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. plant.* **15:** 473 - 497.

Nizeki, H., and K. Oono. 1968. Induction of haploid rice plant from anther culture. *Proc. Jpn. Acad.* **44:** 554 - 557.

Oono, K. 1975. Production of haploid rice plants from anther culture. *Colloq. Int. C.N.R.S.* **193:** 251 - 257.

Quimio, C.A., and F.J. Zapata. 1990. Diallel analysis of callus induction and green - plant regeneration in rice anther culture. *Crop Science.* **30:** 188 - 192.

Raina,S.K., P. Sathish, and K.S. Sarma. 1987. Plant regeneration from *in vitro* cultures of anthers and mature seeds of rice (*Oryza sativa* 1. cv. Basmati - 370). *Plant Cell Reports.* **6:** 43 - 45.

Shen, J.L., M.F. Li., Y.Q. Chen, and Z.H. Zhang. 1983. Improving rice by anther culture. *Proc. of the workshop on cell and tissue culture techniques for cereal crop improvement.* Science press, Beijing, China and IRRI, Manila, Philippines, pp. 183 - 205.

Tamura, S. 1968. Shoot formation in calli originated from rice embryo. *Proc. Japan Acad.* 44: 544 - 547.

Toenniessen, G.H., R.W. Herdt and L.A. Sitch. 1989. Rockefeller Foundation's International network on rice biotechnology. *In:* Review of Advances in Plant Biotechnology 1985 - 88. A. Mujeeb - Kazi and L.A. Sitch, Eds. pp. 265-274.

Wang, C.C., C.S. Sun, and C.C Chu. 1977. Effect of culture factors *in vitro* on the production of albino pollen - plants of rice. *Acta Bot. Sin.* **19**: 190 - 199.

Woo, S.C., and C.C. Chen. 1982. Rice anther culture in Taiwan. Rice Tissue Culture Planning Conference IRRI, Los Banos, Laguna, Philippines. pp. 83 -90.

Yan, C.J., and Q.H. zhao. 1982. Callus induction and plantlet regeneration from leaf blade of *Oryza sativa* L. Sub sp. indica. *Plant Sci. Lett.* **25**: 187 - 192.

Yamada, Y., and W.H. Loh. 1984. Rice. *In:* Handbook of Plant Cell Culture, vol. 3. P.A. Ammirato, D.A. Evans, W.R. Sharp, and Y. Yamada Eds. MacMillan. New York. pp. 151 - 170.

Zapata, F.J., M. Akbar, D. Senadhira, and D.V. Seshu. 1989. Salt tolerance of anther culture - derived rice lines.

Zhaou, C., and H.Y. Yang. 1981. Induction of haploid rice plantlets by ovary culture. *Plant sci. Lett.* **20**: 231 - 237.

Xu shi - huan. 1981. Current status on the anther culture breeding of keng rice. *In:* Studies on anther culture rice breeding. Shen Jin - hua *et al.* eds. 1981.

NOTES

Authors' addresses: M.H. Mendis, A. Hettiarachchi and P. Ganashan, Plant Genetic Resources Centre, P.O. Box 59, Peradeniya; and M.P. Dhanapala, Central Rice Breeding Station, Batalagoda, Ibbagamuwa.

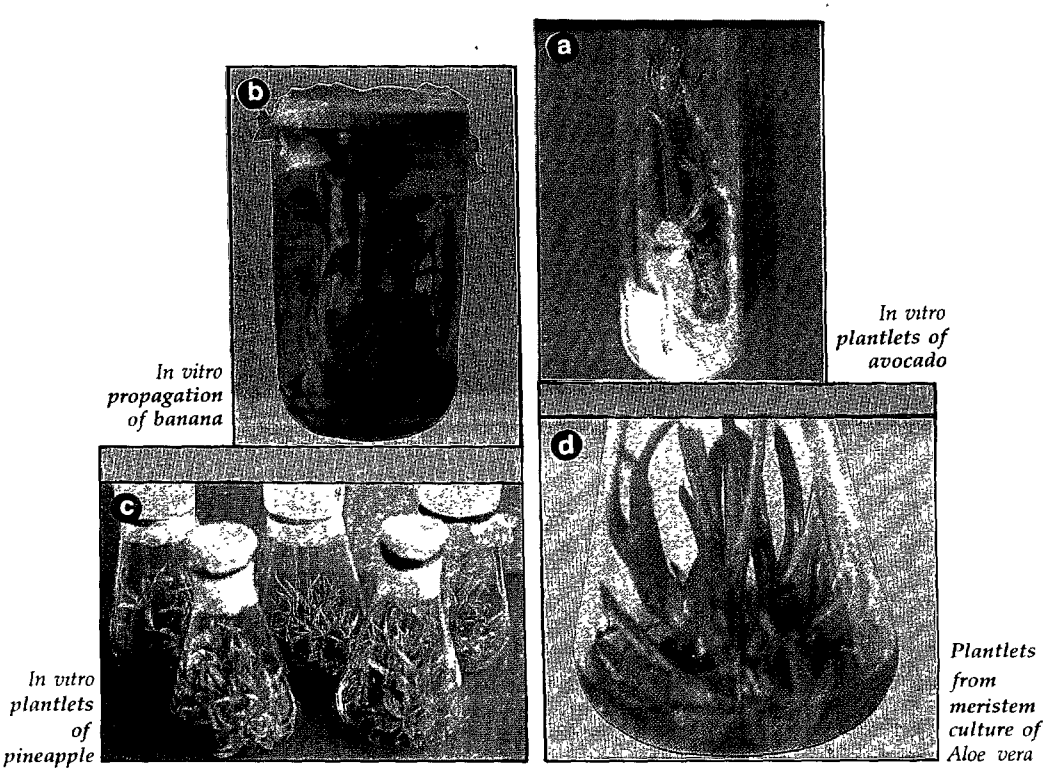


Plate 1. ***Tissue culture propagation of fruits & medicinals***

Application of tissue culture in fruits and some medicinal plants of economic importance

D.P.Rajapakse, A.I.Wickramasinghe, and P.Ganashan

INTRODUCTION

Tissue culture technology plays an important role in crop improvement programmes, in helping to overcome barriers experienced in conventional breeding methods, and in rapid clonal propagation of crops. This technology thus enhances plant breeding and speeds up the release of new and superior clones in a short time in large quantity.

Sri Lanka has a greater potential for the cultivation of many crops due to wide diversity in climate and soil existing throughout the country. Many type of crops such as fruits and other economically important crops which have a good demand in local and export markets can be grown in the island. However cultivation of such crops is hampered due to difficulty in obtaining good quality planting materials. Most of the tropical and semitropical fruits, medicinal plants and forest trees cannot be propagated by seeds due to problems of sterility, heterozygosity and quick loss of viability of seeds. Hence, a suitable propagating material other than seeds has to be selected.

Propagation by tissue culture is performed *via* : (i) enhanced axillary bud breaking, (ii) producing adventious shoots and (iii) somatic cell embryogenesis (Anderson 1980). First two methods are currently applied for many crops and the latter technique which is the most efficient method known so far for vegetative propagation is still in the experimental level.

The concept of tissue culture propagation of plants has four developmental stages (Murashige 1974) :

Stage 1 : Establishment of initial plant material (explant) in culture.
Stage 2 : Multiplication of propagules in culture.
Stage 3 : Rooting of propagules and hardening to survive in soil.
Stage 4 : Planting in soil.

Department of Agriculture is conducting studies on micro-propagation in several crops. Some of the crops tested and the achievements obtained so far are outlined.

MATERIALS AND METHODS

Fruit crops

Banana (*Musa* spp.) cv. Tetraploid, William hybrid, Ambul

Apical meristems aseptically obtained from suckers were divided into four parts and cultured on MS (Murashige & Skoog 1962) medium with 6-benzylaminopurine (BAP). When single shoots were formed they were transferred to MS medium with coconut water (CW) to improve size and vigour of shoots. These shoots were subcultured on same medium supplemented with BAP and CW to enhance shoot multiplication. Individuals shoots were cultured on MS medium with indolebutyric acid (IBA) to produce roots. Rooted plantlets were transplanted in a compost mixture of garden soil, coir dust and cattle manure in pots and acclimatized in green house prior to field planting.

Pineapple (*Ananas comosus*) cv. Mauritius.

Lateral buds aseptically obtained from crown and ratoon suckers were established on modified MS medium with BAP for culture initiation. Growing buds were transferred to same medium with naphthaleneacetic acid (NAA) to form vigorously growing single shoots. These shoots were transferred to liquid medium with BAP and subjected to constant agitation at 70 rpm for shoot multiplication. Shoots were separated and cultured on solid MS medium with IBA to form roots. Plantlets were established in a porous potting mixture of sand, coir dust and cattle manure before field planting.

Avocado (*Persea americana*)

Buds obtained from immature shoots were aseptically cultured on modified MS medium to initiate cultures. After four weeks the healthy cultures were selected and subcultured on different media supplemented with growth hormones (auxins and cytokinins) to induce shoot multiplication. Activated charcoal was added into media as an adsorbent. Shoots at 2-3 leaf stage were transferred to different test media for rooting.

Strawberry (*Fragaria vesca*) cv. Kendall

Meristem domes with 2-4 leaf primodia were established on Boxus medium (Boxus 1974) to produce single shoots. Transfer of these shoots to MS medium supplemented with BAP produced multiple shoots. These shoots formed roots on same medium containing IBA. Plantlets were transplanted in a soil mixture consisting of garden soil, cattle manure and coir dust.

Jak (*Artocarpus heterophyllus*)

Buds obtained from young shoots were aseptically cultured on modified MS medium for culture initiation. Further studies are in progress.

Citrus spp.

Shoot-tip cultures have been initiated and studies are being continued.

Medicinal plants

Komarika (*Aloe vera*)

Shoot meristems were aseptically cultured on solid MS medium to initiate the growth of single shoot cultures. These shoots were transferred to liquid medium supplemented with BAP and subjected to constant agitation at 60 rpm to facilitate shoot multiplication. Shoots were separated and cultured on hormone - free medium to induce rooting. After 3-4 weeks the rooted shoots were transplanted in pots containing a compost soil mixture.

Niyangala (*Gloriosa superba*)

Shoot-tips obtained from young plants were aseptically cultured on B_5 medium (Gamborg's 1968) to enhance single shoot formation. These shoots were subcultured on liquid MS medium supplemented with BAP and IBA and subjected to agitation at 30 rpm for 2-3 months to induce multiple shoot formation. The shoots were separated and cultured on MS medium with IAA for rooting. After 2-3 weeks the rooted shoots were transplanted in pots containing soil and acclimatized in the laboratory under diffused light for 3 weeks before transferring to green house.

RESULTS AND DISCUSSION

Banana

A single banana meristem will produce about 500 shoots in three consecutive proliferations in culture (fig. l). It is possible to obtain this number of shoots during the multiplication stage. These shoots formed roots easily and after acclimatizing for 2-4 months, they were successfully established in the field (Plate 1).

Pineapple

One sucker of pineapple yields over 10 fold increase in shoot production in liquid medium with constant agitation in a period of 4 weeks (fig. 2). These shoots can be easily rooted and have 100 percent field survival rate (Fernando 1986). Field trials conducted at Gannoruwa and Makandura Research Centres indicated that plants derived from tissue culture and from conventional propagation methods could be grown under same field management practices (Plate 1).

Figure 1. Established protocol for micropropagation of Banana *in vitro*.

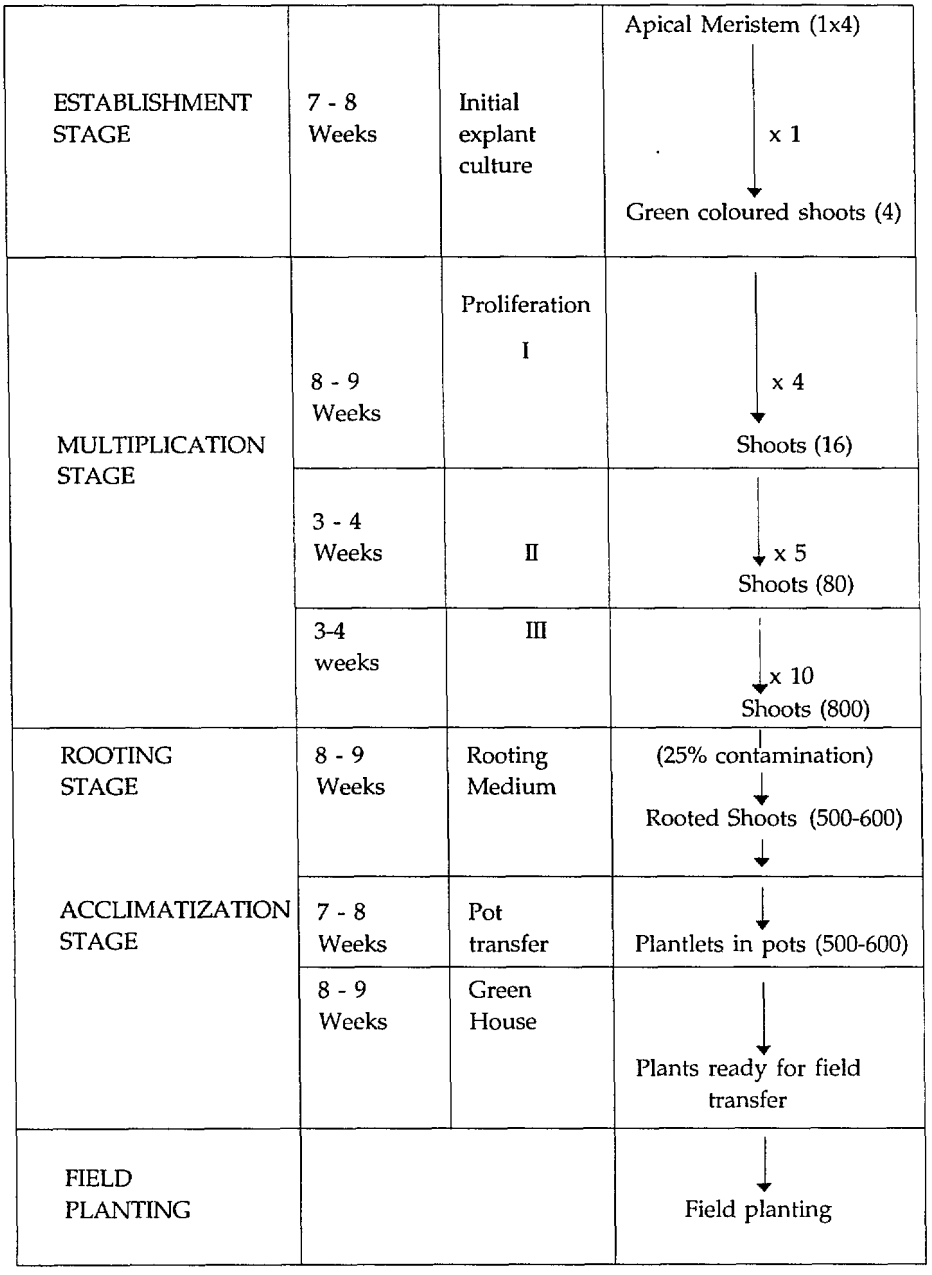


Figure 2. Established protocol for micropropagation of Pineapple (cv. Mauritius) *in vitro*.

ESTABLISHMENT STAGE	3 - 4 Weeks	Initial explant culture	Shoots (25-30 buds) (50% contamination) ↓ Green coloured shoots (4)
	4 - 5 Weeks		(10-15 buds) ↓ Single shoots (10)
MULTIPLICATION STAGE	3 - 4 Weeks	Proliferation I	x 10 ↓ Shoots (100)
	3 - 4 Weeks	II	x 10 ↓ Shoots (1000)
	3 - 4 Weeks	III	x 10 ↓ Shoots (10000)
ROOTING STAGE	8 - 9 Weeks	Rooting Medium	(25% contamination) ↓ Rooted shoots (7500)
ACCLIMATIZATION STAGE	8 - 9 Weeks	Pot transfer	↓ Plantlet in pots (7500)
	8 - 9 Weeks 3 - 4 Weeks	Green house Nursery	↓ Plants ready for Field transfer
FIELD PLANTING			↓ Field planting

Avocado

Healthy shoots with large leaves were obtained when low salt concentrations were used in the initial culture medium. Growth rate of these cultures were slow and stem base expansion was observed in media lacking charcoal. This compound acts as an adsorbent of toxic exudates from the explants and thereby promotes rooting and growth of healthy cultures (Jones 1976). It was observed that rooting was poor and studies are being continued to improve root initiation efficiency (Plate 1).

Strawberry

A single meristem with 2-4 leaf primodia produced 6-7 shoots when BAP was incorporated into the medium. Supplementing with coconut water (CW) produced similar results at lower level (5% v/v) and at higher concentrations (10% v/v) gave marginal increase on shoot proliferation over optimal concentrations of BAP (Wickramasinghe 1988) . These plants showed more than 90% field survival rate .

Komarika

One shoot-tip produced over 40-50 shoots in 6-8 weeks in liquid culture with constant agitation (Plate 1). Shoots formed roots in 4 weeks and after transplanting in pots and acclimatized for 3-4 weeks in the green house, the plants were established in field (Hettiarachchi *et al.* 1992).

Niyangala

Liquid culture system enhanced high shoot multiplication rate and produced 50-80 shoots per culture in about 3 months (Plate 1). These shoots expressed 70% rooting ability and indicated 100% survival rate in soil (Samarajeewa *et al.* 1990).

Tissue culture offers a technology for mass propagation of disease free, uniform size plants in a short time, independant of climatic variations. It facilitates easy production planning and international exchange of plant material to a greater extent.

Formation of genetic off-types in culture which is undesirable in clonal propagation can be eliminated by prevention of undifferentiated callus growth from explants (Scowcroft 1984) and limiting multiplication cycle to three subcultures (Drew 1980) . It is estimated that, for the production of 1, 000, 000 strawberry plants per year *in vitro*, two qualified *in vitro* culture technicians and a worker trained in glasshouse techniques are necessary. Rest of the work can be handled by ordinary workers (Damiano 1980).

Although the initial expenditure in tissue culture propagation is high, when the costs involved in land, irrigation facilities, fertilizing, pest and disease control and labour management are considered, the cost of production of planting materials using tissue culture techniques is comparatively less than other multiplication techniques. Hence this technique can be utilized in clonal propagation of crops to produce large quantity of planting materials.

CONCLUSION

Methodology is established for mass propagation of banana, pineapple and strawberry *in vitro*. In banana, 400-500 plantlets can be obtained from a single shoot-tip in one year and in pineapple, one shoot can be made to yield on the average 7500 plantlets in a year. These plantlets can be grown in the field same as normal plants. It was found that BAP (2.0 mg/l) was optimal for shoot proliferation of strawberry, and coconut water (10% v/v) can replace this costly hormone in enhancing shoot multiplication.

Woody crops by nature, are shy to respond *in vitro* than herbaceous species. (Schall 1987). Hence crops such as avocado and jak requires intensive research studies in order to perfect propagation methodology. Present findings indicate the possibility of using *in vitro* techniques for these crops.

Preliminary findings indicate, that an *in vitro* plantlet regeneration potential of 40-50 in komarika and 30-50 in niyangala could be realized in 5 to 6 months.

References

Anderson, W.C. 1980. Mass propagation by tissue culture. *Proc. of the Conference on nursery production of fruit plants through tissue culture - Application and feasibility - 1980.* U.S. Dept. of Agric., Beltsville, Maryland.

Boxus, P.H. 1974. The production of strawberry plants by *in vitro* micropropagation. *J. Hort. Sci.* **49:** 209-210.

Damiano, C. 1980. Planning and building a tissue culture laboratory. *Proc.of the Conference on Nursery Production of fruit plants through tissue culture - Application and feasibility-1980* U.S.Dept. of Agric., Beltsville, Maryland, U.S.A.

Drew, R.A. 1980. Pineapple tissue culture - unequalled for rapid multiplication. *Queensland Agric. Journal.* pp. 447-451.

Fernando,K. 1986. *In vitro* propagation of mauritius pineapple. *Tropical Agriculturist.* **142:** 7-10

Gamborg, O.L., R.A.Miller, K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell. Res.* **50:** 151-158

Hettiarachchi, A. *et al.* 1992. Unpublished.

Jones, O.P. 1976. Effect of phloridzin and phloroghicinol on apple shoots. *Nature* **262:** 392-393

Murashige, T., and F.Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. plant.* **15:** 473-497.

Murashige, T. 1974. Plant propagation through tissue culture. *Annu. Rev. Plant Physiol.* **25:** 135-166.

Samarajeewa, P.K., M.D.Dassanayake, P.V.Rajapakse, S.D.G.Jayawardena.1990. A study of the application of *in vitro* methods for the propagation of Niyangala (*Gloriosa superba* L.). SLAAS, Colombo.

Schall, S. 1987. La multiplication de lavocatier (*Persea americana* MILL. cv. Fuerte) par microbouturage *in vitro*. *Fruits.* **42 (3)** : 171 - 176

Scowcroft, W.R. 1984. Genetic variability in tissue culture impact on germplasm conservation and utilization. IBPGR Report, Rome.

Notes

Authors' addresses: D.P. Rajapakse and P. Ganashan, Plant Genetic Resources Centre, P.O. Box 59, Peradeniya; and A.I. Wickremasinghe, Central Agricultural Research Institute, Gannoruwa, Peradeniya.

Plant tissue culture in sugarcane breeding

M. Krishnamurthi

INTRODUCTION

Tissue culture was commenced as a tool to study the physiological processes in plant organs, tissues and cells. Later it was found that the independently grown tissues could be induced into somatic embryogeny to produce plants (Gautheret 1939,1982) variously termed as subclones, somaclones or calliclones. Heinze and Mee (1968a, 1968b) found that variable tissues and plants could be obtained from a single donor. Simultaneously Krishnamurthi (1968, internal report) working with *Saccharum officinarum* cultivar pindar, discovered that variant subclones could be obtained for resistance to Fiji disease and downy mildew, *Sclerospora saccharii* Miy. (Krishnamurthi and Talaskal 1974). This discovery triggered the exploration of the potentials of tissue culture to develop variation in the subclonal populations (Larkin and Scowcroft 1981, Scowcroft *et el.* 1987). Since then tissue culture has progressed rapidly in that now it is used for many purposes such as:

- Crop improvement through creation of variation.
- Segregation in intergeneric and interspecific hybrids.
- Rapid propagation.
- Genetic engineering.
- Parasexual hybridization.
- Production of secondary metabolites, edible colours and enzymes.

The techniques of tissue culture and microbiology have now been combined to be used for many of the biotechnological processes such as :

- Energy generation i.e methane from wastes - vegetables and animals.
- Conversion of starch into sweetners.
- Recovery of organic fertilizer.
- Single cell protein.
- Fermentation, fermented foods, and flavours.
- Vaccines.

In this paper we shall confine to the formulation of tissue culture programmes for crop improvement and application of such improvement at farm level to better the

standard of living of the farmer through increased production. The methods of tissue culture shall only be briefly mentioned in relation to the goals established as the procedures are well documented for sugarcane (Krishnamurthi 1974,1977,1981b,1989) and others by various authors present today.

The Administrators' dilemma

Whenever tissue culture or biotechnological research proposals are made, the administrators and financiers become guarded. Naturally, being classical administrators they have many questions, some of which are:

- What has been the success rate historically?
- Could it be an expensive exercise as we do not know much about it?
- Are these show pieces to satisfy the ego of the scientists?
- Is it designed to enable the scientists only to participate in overseas conferences and meetings?
- Do we have enough knowledge and technology?
- Are our scientists trained to undertake the relevant researches?

Perhaps it may not be out of place to answer some of the above questions which will, to a large extent answer many of the questions which usually face the administrators and financiers alike and help the scientists to formulate workable plans oriented to achieving goals.

Historical success rate

Tissue culture like any other science had both successes and failures. The failures were due to lack of understanding of the various plant species, and more so due to poor selection procedures adopted. The successes were rapid where projects were managed appropriately oriented towards achieving results eg. disease resistant varieties in sugarcane (Krishnamurthi and Tlaskal 1974, and Krishnamurthi 1989).

Rapid propagation

This has been the most successful area of tissue culture whereby thousands of plants are produced annually. It is widely applied in horticultural plants (orchids, crysanthimum), commercial crops (bananas, potatoes), trees, and tree crops. This technique could be used extensively in Sri Lanka.

Embryo rescue

This technique is most useful in recovering embryos from interspecific and intergeneric crosses where the embryos normally abort due to embryo-endosperm incompatibilities. The technique can be of great value to the breeders. e.g. sugarcane intergeneric hybridization (Krishnamurthi 1966, internal reports).

Doubling of chromosomes

Haploids from F_1 when doubled through anther culture, will produce homozygous plants thus short circuiting prorammes involving backcrossing for many generations.

Creation of variation

Tissue culture has been used most successfully to obtain variants from single donors through the manipulations of the system to obtain commercial clones especially in vegetatively propagated plants. Sugarcane is a glowing example (Larkin and Scowcroft 1981).

Induction of segregation

This has been a recent discovery by Krishnamurthi and Rao (1990). In this case the intergeneric hybrids are used as donors to spread variations. The subclones from the intergeneric hybrid donors have wide distribution with remarkable degree of heterosis, a feat which is impossible to achieve through normal sexual processes (Krishnamurthi *et al.* 1990).

Haploidy

The success has been limited due to secondary fusion and segregation. Yet it is a useful tool in breeding which should be exploited. The Chinese have used it successfully to obtain commercial cultivars of *Oryza*.

Suspension cultures

Suspension cultures are generally used for screening tissues and cells for resistance to toxins, salinity and other stresses and to obtain protoplasts. This is an area where Sri Lanka may wish to concentrate more.

The capital cost would be about Rs. 750,000/= to establish a laboratory inclusive of building. The recurrent expenditure will depend on the volume of work. A laboratory of this size can produce for example 3000 sugarcane subclones, 50,000 banana plants. 50,000 bananas at Rs. 10/= will give a gross return of Rs. 500,000/= and 100,000 plants will double the income without substantial increase in fixed costs.

As far as breeding is concerned, the 3000 subclones will give at least 300 modified clones of which at least 30 can be tested for commercial potential. This is possible because the starting materials i.e. the donors are already selected clones. To achieve this result through normal breediing will cost six times the value. Above all by working in shifts the laboratory can be devoted to multiple use.

Will the project end as a showpiece ?

The results will speak for themselves as shown by CTC and others this morning and Krishnamurthi (1989), Sreenivasan (1990, personal communications) who have developed many varieties through the system.

Are there enough working details ?

While there is sufficient information and working details on the routine aspects, one must realize that the latest information is always kept confidential till patents are registered and therefore Sri Lanka must embark on advanced researches to be ahead of the industry and the competitors. This will depend on leadership, management, and above all the will and determination of the scientists backed with continuous financing.

Does Sri Lanka have trained scientists ?

Sri Lanka has abundance of skills and intellegentsia which are very poorly managed and guided. It is paramount that research and development should be separated from the civil service into autonomous bodies with freedom of management, fixing of salaries on output basis, and benefits and previleges for the researchers. The whole purpose of Research and Development will be defeated otherwise.

Will the investment payoff ?

Research has low success rates. However those that are successful bring in wealth and happiness to the country at large. It helps to keep the productivity ahead of the competitions, meets the demands of the people, provide insurance for the future.

Having stated the possibilities it is only fair to examine the limitations before we proceed to show what are the alternatives to achieve positive results. From the experience gained in Sri Lanka, some of the constraints may be outlined under the following headings:

1. Precise and clear definition of objectives is necessary and quite often the projects are formulated without any specific aims or goals. It is imperative that the mission must be stated and aims and strategies defined on a time frame basis.

2. Strategies are lacking in most projects, in fact, not at the laboratory level but at the pilot schemes and final implementation stages.

3. The programmes are undertaken quite often only to the point when the ecological trials are carried out and without much thought of how to take the results to the farmer with some exceptions like paddy.

4. Incomplete and unsustained finances emanating from government systems. At other times the resources are spread too thin for it to be effective.

5. The Scientists, generally in their enthusiasm to economise, sacrifice scientific output and quality, e.g. using cheaper and below capacity glassware.

6. The Scientists are not eager to exchange information due to isolation and lack of communications, lack of adequate meetings and conferences.

7. Lack of experience in commercialization of the products.

8. Lack of sustained inputs and continuity due to disruptions caused by many holidays, staff turn over, and down time by the scientists.
9. Lack of appropriate guidance and management of projects.
10. Poor remunerations, lack of incentives and benefits to the scientists.

So far attempts were made to indicate what may be achieved in the world of tissue culture and what may be the constraints faced by the scientists and the administrators. In the following text we shall show how the project can be made successful. As an example of improvement of sugarcane crop, we shall indicate how the projects were formulated and goals achieved with sugarcane. However it must be cautioned that any project formulated must have flexibility to change in midcourse.

Hypothesis

Researchers in Hawaii (Heinze and Mee 1968) found that the sugarcane plants i.e. *Saccharum officinarum* cultivars were chromosomal mosaics. This was one major discovery in the plant cytology. Thus it enabled the author (Krishnamurthi 1966, internal report) to believe that if tissue culture technique is applied to sugarcane, it should be theoretically possible to obtain segregants with different numbers of chromosomes i.e cytotypes. However as subsequent researchers have shown, the subclones were also found to be chromosomal mosaics (Krisnamurthi and Tlaskal 1974).

Aim

With the above in view the aim of the project was to obtain from various commercial or near commercial donors, sub clonal population with modified stable characters required for the production of commercial canes.

Plan Period

Fiji : 1966 - 1972

Sri Lanka : 1987 - 1992

Research Programme

Develop tissue culture techniques for:

- Callus culture.
- Induction of differentiation.
- Induction of roots,
- Transfer to soil.
- Multiplication.
- Screening for various characters.

Management

Sugarcane Research Centre (Fiji) under the sugarcane breeder. Sugarcane Research Institute (Sri Lanka) under the sugarcane breeder.

Laboratory

Basic laboratory facilities with sterile room, laminar flow, preparation room, culture room, and equipment such as balance, pH meter, microscope (stereo-low power), glassware and basic chemicals.

Materials

Various donor clones were used. Some 40 donors were used in Fiji and altogether 18 in Sri Lanka.

Results

Fiji produced the first commercial cane resistant to Fiji disease i.e. *Saccharum officinarum* var. Ono, from *Saccarum officinarum* var. Pindar, in 1974. Since then 22 subclones have been identified for large mill test and the results are awaited. This was delayed due to political unrest and staff turnover of 1987.

At Uda-Walawa two subclones of CO 775 are in stage 6 i.e. advanced stage trial, 500 subclones in disease trial, 1200 in multiplication stage, 1900 in single clumps and 800 in trays and pots.

In Fiji another startling discovery was made with intergeneric hybrid LF 63-75. (Hybrid between *S. officinarum* var. Korpi x *Narenga porphyrocoma*). The subclonal progenies obtained had a very wide distribution for sucrose, fibre, fresh weight, conductivity of juice, stalk height, stalk thickness and yield (Krishnamurthi and Rao 1989).

Commercial clones can be identified and tested for release within seven years against 13 years in a normal programme.

CONCLUSIONS

Tissue Culture is more often misunderstood than appreciated for its potentials and contributions to crop improvement and multiplications. It is essential that goal oriented plans are drawn in time frame with adequate funds, staff, laboratory space, equipment and chemicals. The laboratory should be designed for the needs. It is also essential that academic institutions should leapfrog into future rather than be satisfied with aid and researches undertaken 20 years ago by the advanced countries.

References

Gautheret, R.J. 1939. Culture due Tissue camibial. *C. R. Academy of Sciences.* **208: 118 - 119.** Paris

Gautheret, R.J. 1982. Plant Tissue Culture : The History. *Proc. of Plant Tissue Cell and organ culture.* **5: 7 - 12.**

Heinze, D.J., and G.W.P. Mee. 1968. Tissue, Callus differentiation and regeneration of plants. *Saccharum spp. Agronomy Abstracts.* p. 10.

Heinze, D.J., and G.W.P. Mee. 1969b. Morphogenetic, Cytogenetic and enzymatic variations in *Saccharum spp.* hybrids derived from callus tissue. *American Journal of Botany.* **58: 257 -262.**

Krishnamurthi, M., and Tlaskal. 1974. Fiji disease resistant *Saccharum officinarim* var. Pindar subclones from tissue culture. *Proceedings of the International Society of Sugar Cane Technologists.* **5 (1): 130 - 137.**

Krishnamurthi, M. 1977a. Sugarcane improvement through tissue culture. *Proc. of ISSCT.* **16 (1): 23 - 28.**

Krishnamurthi, M. 1981. Sugarcane improvement through tissue culture and review of progress. *In : Tissue culture of Economically Important Plants.* eds. A.N.Rao, University of Singapore, pp. 70-77.

Krishnamurthi, M. 1982. Disease resistance in Sugarcane developed through tissue culture. *Proc. 5th Int. Cong. of Plant Tissue and cell culture.* **5: 769 - 770.**

Krishnamurthi, M., D.L. Rao, and S. Reddy. 1990. Segregating subclonal population of intergeneric hybrids of *Saccharum :* A new approach to breeding. *In : Proc. of the 6th Int. Cong. of SABARAO,* eds. S. Iyama and Taksda, pp. 417 - 420.

Krishnamurthi, M. 1989. Development of subclonal populations in sugarcane and their genetic and field evaluation for commercial use. Ph.D. Thesis, University of the South Pacific, pp. 340.

Larkin, P.J., and W.R. Scowcroft. 1981. Somaclonal variations - A novel source of variability from cell cultures. *Theoretical and Applied Genetics.* **60: 1917 - 214.**

Scowcroft, W.R., R.I.S. Brettelle, S.A. Rayan, P.A. Davies, and M.A. Pallotta. 1987. Somaclonal variations and genomic flux. *In: Plant Tissue and Cell Culture.* Green, C.E., D.A. Sommers, W.P. Hackett and D.D. Biesboer, eds.,pp. 275 - 286.

Notes

Authors' address: M. Krishnamurthi, Sugarcane Research Institute, Udawalawe.

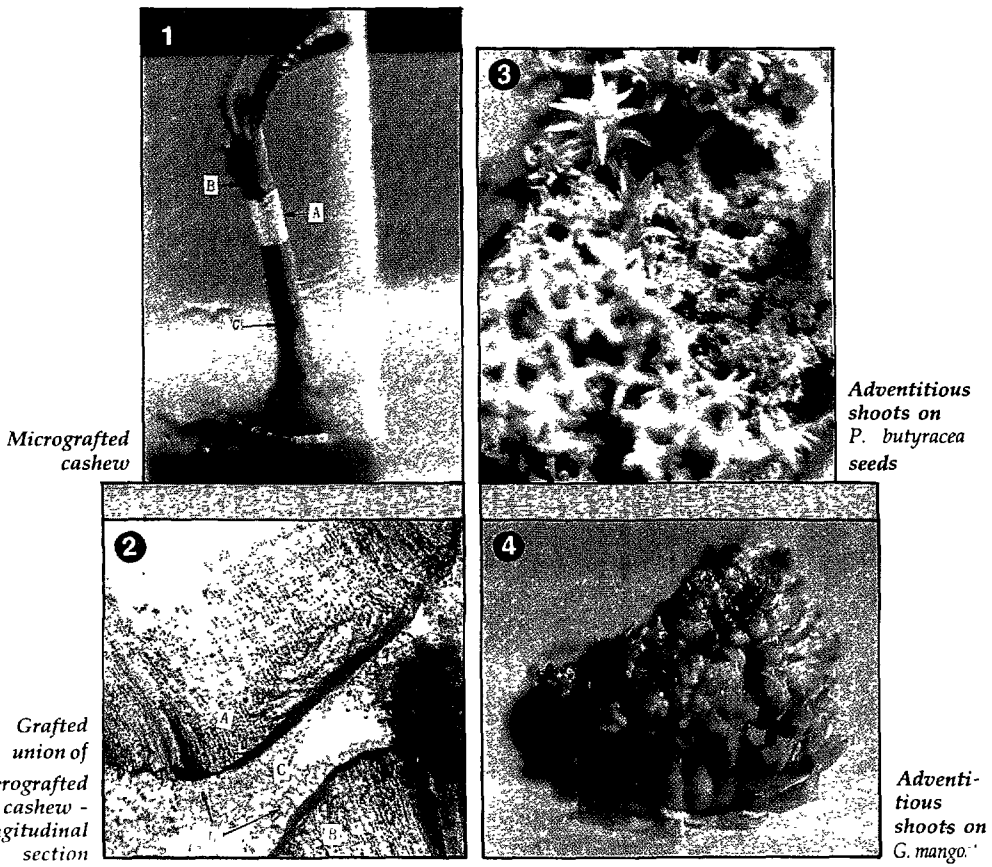


Plate 1 - 4. In Vitro ***micrografting***

1A: aluminium foil tube. 1B : Scion. 1C : root stock

2A: Scion. 2B : root stock. 2C : newly formed cells

2D: differenciating conducting tissues

In vitro micrografting in cashew and mangosteen

S.M.S.D. Ramanayake and A. Kovoor

INTRODUCTION

Cashew can be recommended as a crop for the dry zone because of its hardy drought and wind resistant nature. It has become an important cash crop due to the high international demand for its kernels. Therefore providing improved planting material to establish plantations for the cashew industry is an urgent requirement. Being heterozygous and outcrossing, improvement should centre around tree selection and vegetative cloning. Considerable variation in nut quality and yield have been reported (Northwood 1966). Ample scope is therefore available for selection of superior trees. Vegetative methods of cloning by conventional methods have not produced consistent results due to difficulty in rooting. Grafting to a seedling root stock of the same species or a related species could overcome this problem. By selecting a species with an even better root system, further improvement could be achieved. Grafting to a seedling stock could also induce a return to the juvenile state in vegetative buds of mature tees (Huang *et al.* 1990). This could induce budding and rooting which is difficult to achieve in mature trees.

Anacardium microcarpum Ducke is a wild species native to Brazil (Johnson 1973) and could be used as a root stock to improve cashew. Generally the closer the taxonomic relationship between root stock and scion the more easily they fuse (Fuji and Nito 1972). Homoplastic grafts of cashew by conventional methods are difficult. Therefore, *in vitro* micrografting was tried as it is more amenable to manipulations than conventional methods. The root stock and scion are not subjected to water stress and can be maintained in a more precisely controlled chemical and physical environment (Parkinson and Yeoman 1982). This would help to enable fusion in difficult grafts.

Mangosteen *(Garcinia mangostana L.)* is an underutilized fruit tree with a promising economic value. It requires a moist climate, and is found only in limited areas of the wet zone. It is propagated by seeds and takes 8-10 years to bear. Seeds are apomictic (Lim 1984). Seedling survival is low and tree establishment during the first few years is difficult due to slow growing root system. The root system is reported to consist only of the tap root and usually lacks lateral roots and root hairs. Although various asexual methods, including bud grafting, have been tried to resolve the problem of poor root development and low seedling survival, none have been successful (Almeyda and

Martin 1976). *In vitro* micrografting to a species belonging to the same family *Clusiaceae*, with a better root system is a method which could be tried. Among the many species of *Garcinia* available, *G. morella, G. benthami, G. xanthocarpum* as well as *P. butyracea* which are drought resistant species, are being used as root stock in the experiments on *in vitro* micrografting.

MATERIALS AND METHODS

Cashew

Establishment of the root stock: Mature green nuts and dry nuts were collected from Puttalam and Lunuwila during the cashew season. These were stored in a cold room at 20°C and used for about 6 months. A few dry nuts of *A. microcarpum* Ducke, obtained from Fortalezza in Brazil were also used. The nuts were opened and the embryonic end with part of the cotyledon was extracted and surface sterilized. The seed coat was removed and the primary axis with a small part of the cotyledon was explanted in a basal MS medium (Murashige and Skoog 1962) with 2% sucrose and 0.5% agar. After one week the germinating embryos were transferred to a basal MS medium supplemented with 10% coconut water, 5µm asparagine, 1.5mgl^{-1} BAP, 2% sucrose, 0.2% activated charcoal and gelled with 0.6% agar. They were subjected to a 12 hour photoperiod.

Establishment of Scion: Actively growing sprigs were collected from mature trees around Kandy, Lunuwila and Puttalam. Different methods of surface sterilization and fungicide treatment were carried out before the terminal buds and nodal segments were cultured to obtain axenic material. Shoot tips of germinated seedlings were induced to produce axillary buds as described (Ramanayake and Kovoor 1992).

Micrografting: Axenic cultures of vegetative buds from mature cashew trees were not obtained. The technique of micrografting was therefore developed using terminal and induced axillary buds of *in vitro* germinated seedling material as scions in grafts. The root stock was the decapitated *in vitro* seedling of *A. occidentale* or *A. microcarpum*. Two to three week old seedlings bearing at least one open leaf was used. The following experiments were conducted.

Methods of decapitation of root stock and placement of scion on it: Root stock was decapitated to remove all leaves with a flat horizontal, oblique, wedge-shaped cut or slit and the scion base shaped to fit into these.

Method of securing scion on stock: Two methods were tried. Sterilized mixture of lanolin and water in a 1:1 (wt/wt) ratio to form a paste was applied to the freshly cut ends of stock and scion which were then placed together. Sometimes a sterilized aluminium foil tube was placed tightly around the stock and scion junction (Plate 1).

Prevention of browning of cut ends: A few drops of a sterilized solution containing the following substances were applied on the freshly cut surfaces.

- 150 mgl^{-1} ascorbic acid + 150 mgl^{-1} citric acid
- 150 mgl^{-1} ascorbic acid + 5 mgl^{-1} IAA + 2mgl^{-1} kinetin
- 150 mgl^{-1} ascorbic acid + 0.1 mgl^{-1} zeatin
- 150 mgl^{-1} citric acid + 0.2 mgl^{-1} zeatin + 0.1 mgl^{-1} GA3

Pre-treatment of scion: The vegetative buds to be used as scions were placed in the following media for 2, 4 or 7 days before micrografting.

- MS liquid medium + 2% sucrose + 0.01 or 0.1 mgl^{-1} zeatin
- MS medium +2% sucrose + 1% PVP (44,000 mol wt.) + 5 or l0mgl^{-1} IAA gelled with 0.7% agar
- MS medium + 2% sucrose + 5 or 10 mgl^{-1} IAA gelled with 0.7% agar.

Media for growth of grafted seedlings: Basal MS media supplemented as follows were used.

- 5 µm asparagine + 10% coconut water + 1.5 mgl^{-1} BAP + 2% sucrose + 0.2% activated charcoal + 0.7% agar.
- 5 or 10 mgl^{-1} NAA + 2% sucrose + 0.2% activated charcoal + 0.7% agar.

Mangosteen:

Establishment of root stock: Mature or ripe fruits of *G. morella, G. benthami, G. xanthocarpum and P. butyracea* were collected from the Botanical Gardens, Peradeniya. The seeds were extracted and surface sterilized and explanted in a basal MS medium with 2% sucrose, 0.7% agar supplemented with BAP (5mgl^{-1} or l0mg l^{-1}) or without BAP.

Establishment of the scion: Mature green or ripe fruits of mangosteen were collected, seeds extracted and surface sterilized. They were explanted in the same media used for the establishment of the root stock.

Micrografting: Rooted shoots of *P. butyracea* were decapitated to remove all leaves and shoot tips of germinated *G. mangostana* were placed on this. The scion was secured with lanolin paste after a few drops of a solution of ascorbic acid and citric acid at 150mg^{-1} were applied on cut ends of root stock and scion.

RESULTS AND DISCUSSION

Cashew

Establishment of the root stock: The embryos started to germinate in about three days with radicle elongation. The medium browned due to exudation of phenolics. The browning was enhanced when nuts stored for a period of over three months were used.

On transfer to a new medium after a week, further development with elongation of hypocotyl followed by plumule development took place. The embryos of nuts stored for over four months showed reduced germination and vigour with a high percentage of abnormal seedlings. Micrografting was not successful with them.

Establishment of scion: The different methods of surface-sterilization and fungicide treatments tried were not effective in the establishment of axenic cultures to induce axillary buds from mature tree explants. The presence of a systemic fungus was observed in all trees examined. Experiments to produce axenic cultures are progressing.

It was possible to induce development of axillary buds in decapitated shoot apices of *in vitro* seedlings (Ramanayake and Kovoor 1992). The technique of micrografting was practiced using these axillary and terminal buds.

Micrografting: Seedlings freshly decapitated above the cotyledon attachment were used as root stock. An initial problem was the displacement of the scion due to movements caused by turgor or the formation of callus in between. Table 1 shows that the percentage of micrografts where the scion was displaced decreased from 76% to 51% when the scion was placed in a slit in the leaf axil.

Table 1 : Effect of grafting method and treatments observed one month after micrografting in Cashew

Grafting Method/ Treatments	Total No. of Micro-grafts	% with displaced or browned scion	% fresh green scions	% developed scion	% con-tamination
Horizontal cut with Lanolin application	26	76	8	4	11
Scion placed in leaf axil & AIK treatment at cut ends	79	51	26	5	11
Use of foil tube, C2G & MS-IAA treatments	30	33	1	6	63
Use of foil tube, C2G, IAA & rooting medium	7	28	–	57	14

AIK : Mixture of 150 mgl^{-1} ascorbic acid + 5mgl^{-1} IAA + 2mgl^{-1} kinetin.

C2G : Mixture of 150 mgl^{-1} citric acid + 0.2mgl^{-1} zeatin + 0.1mgl^{-1} GA3

MS-IAA : Basal MS supplemented with 2% sucrose and 5-10mgl^{-1} IAA

The petiole tended to support the scion. The displacement was reduced to a further 28% when an aluminium foil tube was placed around the junction of scion and root stock. It was possible to shape the tube to fit the stem and hold the scion firmly. Lanolin paste was not an effective method of holding the scion on the stock. Although lanolin is miscible with water it is possible that solvent movement across it could be hindered. Water movement however took place because the scions that did not fuse remained fresh for periods of over two months. Mosella Chancel (1979) reported the use of an elastic strip as a mechanical support in micrografts.

Initially browning of cut ends due to formation of polyphenols by oxidation prevented fusion in grafts. Martinez (1979) reported that poor micrografting was linked to oxidative browning. Substances such as ascorbic acid, citric acid, thiourea, cysteine, DIECA etc., are used to reduced browning. In our experiments both citric acid and ascorbic acid reduced browning. The combination of citric acid, zeatin and GA3 was the best out of all treatments tried.

A pretreatment of the scion was necessary to bring about fusion. The vegetative buds when placed in a basal MS medium with 5 to mgl^{-1} IAA and 2% sucrose for one week appeared to be the best. After micrografting the plant was placed in a medium which enhanced rooting. MS supplemented with 5 mgl^{-1} NAA, 0.2% charcoal, and 2% sucrose was necessary for the development of the scion into the shoot system.

An interaction between all these treatments during the grafting procedure may have taken place to bring about successful fusion. Parkinson and Yeoman (1982) reported that IAA and kinetin were necessary for the formation of vascular connections in the new tissues formed between scion and stock. IAA alone in our experiments brought about the formation of such vascular connections.

A microscopic examination of longitudinal sections of well developed micrografted plants showed that vascular connections differentiated (Plate 2) among the newly formed cells between the scion and stock.Although the technique of micrografing in cashew has been developed using seedling material as scion, its practical application would be advantageous in the use of vegetative buds of selected trees as scion in micrografts.It was not possible to micrograft cashew on *A. microcarpum* because the number of *A. microcarpum* seeds available was limited. Further *A. microcarpum* was used during the initial stages of the experiment before all the requirements for successful grafting were determined. A few plants of *A. microcarpum* have however been established in soil and there is the future possibility of using them in micrografting with cashew.

Mangosteen:

Establishment of the root stock: Seeds of *G.morella,* and *G.xanthocarpum* were contaminated with fungus. Each seed germinated to produce a single shoot in hormone free medium. A few multiple shoots developed in the presence of BAP, but they all

succumbed to the fungal contaminant eventually. Therefore, surface sterilization techniques need to be improved. *G. benthami*, and *P. butyracea* germinated in hormone free medium. Some of the *P.butyracea* seeds formed a large number of adventitious buds which later developed into shoots on the seed surface (plate 3). In some seeds these, shoots formed only on one side of the seed and a few of them developed roots. The number of shoots per seed showed a wide range from 0 - 200 in both 5 and 10mgl^{-1} BAP. This indicated physiological differences in seeds taken from the same fruit as well as seeds of the same age. A similar variation was seen in seeds of *G.benthami* which germinated in hormone free medium or developed multiple shoots at one end of the seed in the presence of BAP. Induction of rooting of shoots of these species need to be carried out.

Establishment of the scion: Seeds established in hormone free medium germinated to form a single seedling per seed. In the presence of BAP, adventitious buds which developed into shoots formed on the seed surface (Plate 4). The difficulties encountered in the establishment of scion by using mature tree explants is overcome as seedling material is easier to decontaminate and more responsive to growth regulator treatments than mature tree explants. Further the seeds being apomictic, adventitious shoots induced from them are of the same genotype as the mother plant.

Micrografting: There was exudation of latex and browning of cut ends due to formation of tanins in the grafts. The scion turned brown within a week. Further experiments are needed to reduce latex exudation and formation of phenolics and facilitate fusion of scion and stock.

CONCLUSION

Micrografting of cashew on cashew using seedling material was possible when a pretreatment of IAA at 5-10 mgl^{-1} was given to the scion. Oxidative browning of cut ends was reduced with citric acid, zeatin and GA3 mixture. Mechanical support of an aluminium foil tube enhanced fusion. These methods have next to be applied to the selected mother tree explants to make it into a useful technique in improving cashew.

A number of adventitious buds could be induced on seeds of mangosteen. These could be used a scions in micrografts as they are of the same genotype as the mother tree. Adventitious shoots induced on seeds of the root stock species need to be rooted. Experiments to reduce latex exudation and formation of phenolics at cut ends are necessary to enable fusion of micrografts.

References

Almeyda, N., and F.W. Martin. 1976. The mangosteen. *In*: Cultivation of neglected tropical fruit trees with promise. Agricultural Research Service, US Dept. of Agriculture. Nov. 1966, 1-16p.

Fuji, T., and N.Nito. 1972. Studies on the compatibility of grafting of fruit trees. Callus fusion between root stock and scion. *J.Japan Soc. Hort. Sci.* **41(1):** 1-10.

Huang, L.C., R.Van Gundy, and T.Murashige. 1990. Rejuvenation of trees by grafting and its possible mechanism. In:*Abstracts of proceedings of the 7th Int. Conf. on Plant Cell and Tissue Culture.* Amsterdam, June, 24 - 29, 1990.

Johnson , D . 1973 . The botany origin and the spread of the cashew . *Anacardium occidentale* L. *J. of Plantation Crops.* **1:** 1-7.

Jonard, R. 1986. Micrografting and its applications in tree improvement. *In:Biotechnology in forestry and agriculture.* Bajaj, Y.P.S. eds.

Lim, A.L. 1984. The embryology of *Garcinia mangostana* L. *(Clusiaceae).* The Gardener's Bulletin, Singapore. **37:** 93-103.

Martinez, J.E. 1979. Technique de greffage in vitro d'apex appliquees a letude de incompatibilities au greffage chez diverses especes fruitieres du genre Prunus. Thes 3 Cycle, Agron Phytotech, USTL Montpellier, 151 p.

Mosella - Chancel, L . 1979. L'utilisation de l'apex caulinaire comme moyen d'elimination de 2 types de virions chex le pecher. Thes D Ing. Sci. Agron. Phytotech USTL, Montpellier, 215 p.

Murashige, T., and F.Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* **15:** 473-497.

Northwood, P.J. 1966. Some observations in flowering and fruit setting in the cashew *Anacardium occidentale* L. *Trop. Agric.* **43:** 35-42.

Parkinson, M., and Yeoman, M.M. 1982. Graft formation in cultured explanted internodes. *New Phytol.* **91:** 711-719.

Ramanayake, S.M. S.D., and A.Kovoor. 1992. *In vitro* propagation of cashew (*Anacardium occidentale* L.) a tree crop for dry zones. Proceedings of the Third Regional Workshop on Multipurpose Trees, May 2-3, 1992, Kandy (in press).

Notes

Authors' address: S.M.S.D. Ramanayake and A. Kovoor, Institute of Fundamental Studies, Hantane, Kandy.

Discussion

Session I: Generation of Technology

Question - Dr. C.R. Panabokke: I have some concern regarding the usefulness of rapid multiplication of perennial crops, especially their adaptability with regard to the year to year variation in environment. A well known example is the oil palm disaster in West Africa.

Answer - Dr. M.H. Mendis: In the case of oil palm, first a callus was established from which the plants were regenerated. Plants regenerated from callus exhibit genetic variation. That is why they got variants. Most probably many of them would be not fertile. As regards cashew, the shoot multiplication is from axillary buds. Axillary buds, will not generate much genetic variation, may be 1 per cent or less.

Comment - Dr. M. Krishnamoorthy: What tissue culture has taught us is that the classical belief, that 2n numbers, the number in all the cells, is no more true. If you carefully count the chromosomes, you will find that most plants have chromosomal mosaics. They seem to stabilize only at meiosis. But even there, we do not know when the pollen is produced, whether all the pollen has the same genetic material in it. Therefore the seedling populations, when they are produced, have a certain amount of variability in them. When we do rapid propagation through axillary buds, we normally expect less variation, because we start from a more specific material. As Dr.Mendis said, nearly 99 per cent will be fairly good material. This is borne out in many crops, particularly orchids, where lot of work has been done and variations observed are very small.

Q - Dr. C. Kudagamage: In the conventional type of breeding, screening for resistance takes very long time at CRBS Batalagoda and CARI. Are there techniques in tissue culture to help incorporate disease or pest resistance in breeding lines?

A - Dr. M.H. Mendis: Yes, there are methods, but presently we don't have sufficient staff to carry out all these experiments. In the meantime PGRC is supporting the present plant breeding programme by doing embryo rescue work and also by fixing characters in a very short time by anther culture?

Q - Dr. S.M.C. Subasinghe: How long will it take to make available pineapple planting materials to growers in substantial quantities through tissue culture?

A - Ms. D.P. Rajapakse: We can produce 7500 plants within one year from one shoot tip. By repeating the multiplication cycle, the number of plantlets can be increased.

Q - Dr. M. Sikurajapathy: With IRRI going for biotechnology in rice, would it not be important or necessary to determine what priority areas in Sri Lanka should be looked into? Do we need to spend resources, manpower, time etc., repeating work done elsewhere?

A - Dr. M.H. Mendis: IRRI does mostly basic research and we do not want to do the same type of research. We have to do is what is relevant to us. But we have to develop capability to adapt and apply technology generated elsewhere.

Q - Dr. P. Arulpragasam: The scions used in grafting experiments in cashew are from seeds and hence will be heterozygous. Would the character not be known till the grafted plants come to bearing?

A - Ms. D. Ramanayake: This was done to get the technique right. We used the seedling material first because it is easy to work with seedling materials. Mature tree materials do not respond easily to tissue culture. First we use seedling material and then we modify the technique for mature trees. This is the initial stage of the experiment.

Q - Mr. M. Agalawatte: Can the tissue culture technique be picked up by A) private sector nursery men, b) the farmer? Does the Department of Agriculture have facilities to provide materials necessary to meet the demand?

A - Dr. M.H. Mendis: At PGRC, we do not have facilities for large scale multiplication and therefore we cannot provide planting materials on a large scale. But the technology can be given.

C - Dr. Krishnamoorthy: May be I can make a little comment on that? There are certain technologies which can be given to the farmers but others are little beyond the capacity of the farmers. But where a businessman or a business house is concerned, the bulk of the technology can be given provided they are willing to pay for the development cost. Most often what happens is that the business house wants to get everything free and make money while the research institutions need money to run further projects. For instance, in Thailand the potato seed material imported from Holland is cleaned, micro tubers or propagules are produced in test tubes and then established in trays to produce very uniform 2g size tubers. This is a technique of producing seed which the commercial houses can pick up and do.

Q - Dr. Sriyani Peiris: In general, micropropagated plants show high vigour, 40- 50 per cent over the normal. Have you observed this in your field trials of banana?

A - Ms. Asoka Wickramasinghe: Yes.

C - Dr. Sriyani Peiris: I conducted field trials with tissue cultured banana and I have found better yields, more sucker production and earlier fruiting in tissue cultured banana.

Overview

Session I: Generation of Technology

It was evident from the presentations made that scientists are certainly working on some of the priority crops of the Department of Agriculture, like rice and certain fruit species set by CARP. Sugarcane, which does not fall under the purview of Department of Agriculture, is also a priority crop for our country.

A good point to note is that, relevant researches are conducted in different institutions in terms of our crop prioritization. However, the priority areas that we should focus in biotechnology must be decided. Most of the presentations were limited to micro propagation, specifically to tissue culture. But, the title "The Application of Biotechnology" is a very vast term and certainly, we are moving towards a wider research agenda.

In the principal crop rice, the problem was highlighted in terms of self sufficiency which has eluded us for nearly 20 years. Though production has increased substantially the population is rapidly growing and rice production has also reached a plateau. Breeders of International Agricultural Research Centres and National Agricultural Research Institutions are concentrating in different ways, trying to break through the yield plateau or the yield barrier. Certainly, in the case of rice breeding, biotechnology offers many advantages in terms of breaking down the genetic linkages through biotechnological mechanisms. It is difficult to cross the japonica varieties and "wild" species with indica varieties to obtain hybrids due to the complex traits that are associated with it. However, in terms of shortening the time scale in breeding, from the conventional 6-7 years, perhaps to one year or shorter period is certainly an advantage in this technique.

Therefore, the move towards using biotechnology in rice should be fruitful. But here again, the break through will come as suddenly as the one in the physiological changes that took place in breeding the miracle rice of the 1960's. So, from the green revolution we might move to gene revolution where, we have an interaction with a wider group of scientists in the arena in order to be successful.

In the case of the fruits, shortage of planting materials in the species that are identified for development has been highlighted. Therefore, in order to increase the production of planting materials, simple tissue culture mechanisms are being developed, tried out and are being experimented with. In order to get the planting materials in quantities as well as at price levels the growers desire, the initiation of tissue culture techniques that have started in the DOA should be encouraged. There must be wider agenda in order to develop the process to accelerate these techniques.

When we consider the issues that were raised from these presentations, self sufficiency in rice has been identified and the breakthrough that is expected from biotechnology is to overcome the present yield plateau and increase yields necessary to feed the growing population. It has been predicted that the rice yields can be doubled by the use of biotechnological mechanisms. The breakthrough can only be attained sometimes in the future.

This is also true with other crops. Can we go on expanding large scale of the sugarcane plantations, in the land available, which has been identified for sugarcane due to the water regimes as there is a competition for the same land for rice production. This has become a contentious issue. As a consequence, if land becomes the limiting factor for expansion, certainly we have to move further ahead in yields. In the case of sugarcane development, this would certainly be the objective. In addition to the yield increases we like to achieve utilising biotechnological methods to the principal crops like rice, incorporation of resistance to pests and diseases will bring about lot of benefits.

The supply of planting materials of the desired varieties in quantities needed for expanded thrust of production for export, is one of the principal issues faced by the DOA and MAD&R. One aspect is the shortage of planting materials of the varieties that export oriented entrepreneurs wishes to introduce or import. This being so, one method is to develop tissue culture technique for mass production of quality planting materials. Therefore, this particular issue also will be dealt with the expansion of the technique.

The other aspect in relation to issue raised is in regard to cost of biotechnology. Is it cheap or expensive? As pointed out by Dr. Krishnamurthy, it need not be expensive. The existing technology can be modified and developed further by much more simpler approaches to the crop in order to bring about a breakthrough that is necessary.

Another important issue here is the management aspects of biotechnology. In Sri Lanka, so far, there is no concensus on the biotechnology thrust or biotechnological approach to crop production or crop improvement. We hope that from the outcome of this workshop, some kind of recommendations will be forthcoming to develop a national consensus. So that the CARP, within its own mandate, will be able to prioritize as to exactly on what crops and what areas we should focus our development of biotechnology which also means, in what areas we should train our future biotechnologists. Without that kind of an agenda, recommendations or consensus, it will be difficult for the organization at apex level to give the kind of support or direction that it should give in generating and accelerating the technology which should be applied, which is the subject of the next session.

Prof. Y.D.A.Senanayake
Discussant

SESSION II

APPLICATION OF TECHNOLOGY

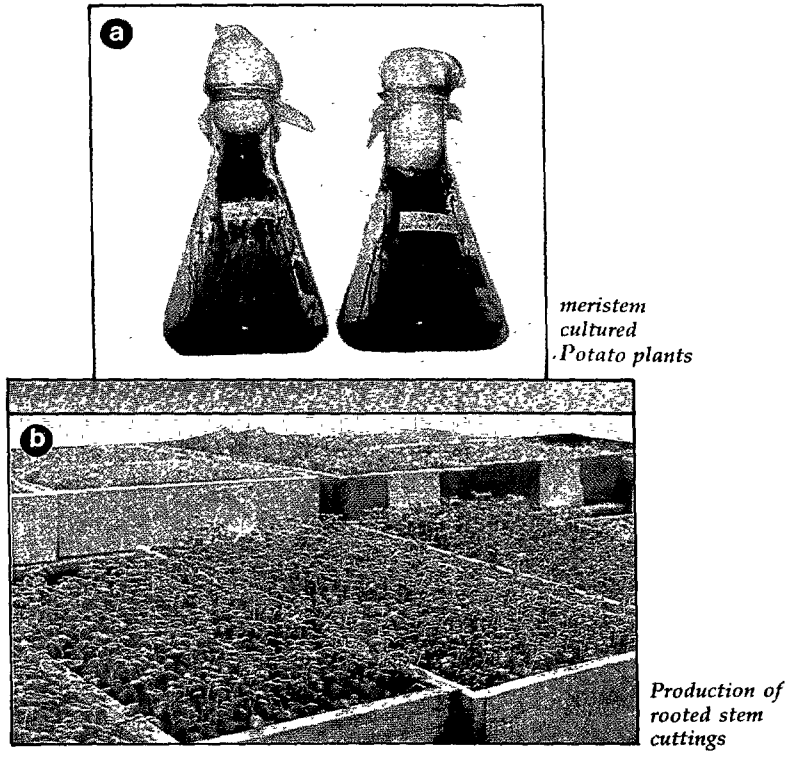


Plate 1. ***Rapid multiplication of potato***

"Basic" seed potato production through rapid multiplication technique

P.W.S. Mallika Samarasinghe

In Sri Lanka, potato is grown over an area of about 7000 hectare. Annual seed potato requirement is nearly 15,000 tons, of which over half is produced by the farmers themselves (Table 1). The conventional method of multiplication of planting materials for potato is by the use of tubers. Main disadvantage of the this mehtod is that the seed potatos produced by planting tubers are not 'clean' and found to be of inferior quality. The conventional method of certified seed production in government seed farms is through multiplication for 2-3 generations of the imported seeds which are normally in the G_5 or G_6 generations. This has resulted in high disease content in the certified seeds that have been produced. Seed potatoes are the most costly single input in potato production (Table 2). Demand for alternatives to the costly and often unavailable good quality seed tubers is increasing in Sri Lanka. A new system has been established for producing healthy seeds based on systematic virus testing and *in vitro* rapid multiplication of virus free planting materials. This technology can be introduced to farmers successfully to produce high quality seed on their own at low cost to meet their seed requirements. From the response received so far from the cultivators for this technology, it is evident that Rapid Multiplication Technology (RMT) needs no "push" by the Department of Agriculture (DOA), instead there is a "pull" by the farming sector in Sri Lanka. Action is also being taken by DOA to extend this technology to the upcountry government farms so as to produce "high grade" seed within the country.

Present Status of the Rapid Multiplication Technology (RMT)

In this scheme, meristem culture, *in vitro* propagation, production of mother plants and production of rooted stem cuttings are presently carried out by the Regional Agricultural Research Centre (RARC), Bandarawela.

Use of RMT in government seed potato production scheme

Rooted stem cuttings produced in the aphid proof net house in RARC, Bandarawela are supplied to 3 net houses in seed potato farms at Pedro, Udaradalla and Sita Eliya to produce pre basic potato seeds. These tuberlets are transferred and planted in open field at Pedro potato seed farm and remultiplied for two or more generations to produce basic seeds. The basic seeds are distributed to other up-country potato seed farms for the production of foundation seeds. The schematic diagram of the proposed seed production scheme is shown in Fig 1.

Table 1: Cultivated Extents and Seed Potato Requirements

District	Cultivated extents (ha)	Seed requirements (MT)			Outside seed sources	
		Own seed	outside source	Total	DOA (MT)	Private Traders (MT)
Maha						
N, Eliya	1000	1000	1000	2000	–	1000
Badulla	1200	300	2100	2400	1400	700
Jaffna	500	–	1000	1000	1000	–
Others	50	–	100	100	100	–
Sub total	2750	1300	4200	5500	2500	1700
Yala						
N' Eliya	1200	1000	1400	2400	1200	200
Badulla	3400	6000	800	6800	–	800
Jaffna	–	–	–	–	–	
Others	–	–	–	–	–	
G. Total	7350	8300	6400	14700	3700	2700

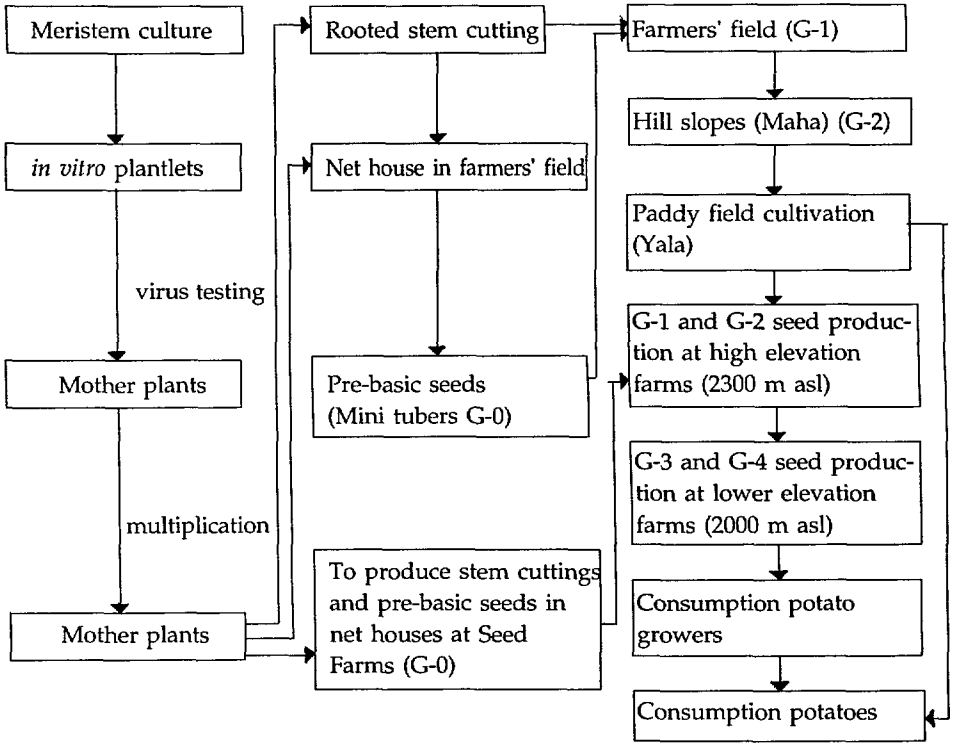
Table 2 : Approximate cost of cultivation of potato / ha

Seed potato (2.5 tons/Rs.50. / kg)		Rs. 1,25,000.00 (60%)
Fertilizer (organic & inorganic)	Rs. 27,000.00	
Agro - chemicals	Rs. 5,000.00	
Labour 650 mandays	Rs. 48,750.00	Rs. 80,750.00 (40%)
Total Cost		Rs. 2,05,750.00 (100%)

Average yield	= 19 tons/ha
Farm gate Price	= Rs. 24/Kg
Gross income	= Rs. 456,000/=
Profit	= Rs. 250,250/=

Cost of production / Kg = 205,750/19,000 = Rs. 10.82

Figure 1. Schematic diagram of government-initiated seed potato production using RMT in Sri Lanka.



Under this scheme in June 1992, approximately 15,000 rooted stem cuttings of cultivar 'Desiree' were issued from RARC, Bandarawela to 3 net houses in seed farms for the first time. A total of 43536 pre basic (Go) tuberlets (1017 Kg) was produced in an area of 225 sq.m. (Table 3).

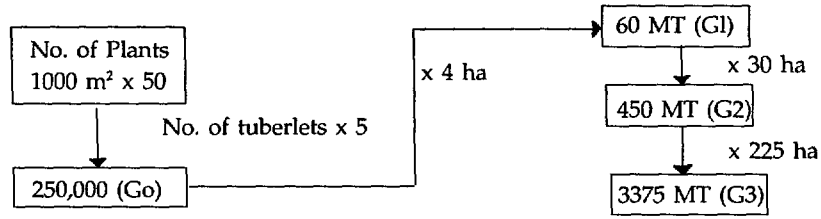
Table 3: Pre basic seed production of cultivar Desiree in seed potato farms in June 1992

Farm	Area planted (Sq.m.)	Number Planted (R.S.C.)	Yield	
			No. of tubers	tuber wt. (Kg)
Sita Eliya	75	4496	14024	300
Pedro	75	5403	18168	504
Udaradella	75	5074	11344	213

* R.S.C. = Rooted Stem Cuttings

At a conservative estimate of the rate of multiplication of 1:5, it is estimated that the construction of net houses in farms with an effective area of 1000 m^2 can produce sufficient tuberlets from stem cuttings, which after two more multiplications can be field planted in 225 ha to yield 3375 tons of foundation seed (Table 4).

Table 4: Estimated basic seed production in net house area of 1000 m^2



Use of RMT in farmers fields

RMT technology was introduced to selected progressive farmers in Badulla district. Each farmer was issued 2000 rooted stem cuttings in 1990/1991 (Table 5). The cultivars distributed were Desiree, Sita and Krushi and extension staff of Uva provincial council co-operated in the demonstrations. Technique of growing rooted stem cuttings were demonstrated to the farmers before the planting materials were issued.

Table 5: Basic seed production in farmers fields

Season	No. Farmers	No. RSC issued	Basic seed production	
			No. tubers	Tuber Wt. (Kg)
Yala 1990	8	15,490	68,317	718.3
Maha 1990/1991	7	13,800	70,001	605.0
Yala 1991	5	9,775	56,926	442.0
Maha 1991/1992	12	17,958	78,582	1160.0

The 2000 rooted stem cuttings were grown in a 40m^2 plot with a spacing of 15 x 15cm, and sited close to the cultivators house to ensure better management. These cuttings yielded over 10,000 tuberlets which were sufficient to plant 0.14 ha at the standard recommended spacing of 60 x 25 cms. To plant an area of this size with normal seed tubers, a farmer requires 280 Kg of seed valued Rs. 16,800/= to Rs. 22,400/=, at the present price of Rs. 60/= to Rs. 80/= per Kg of seed potatoes.

The detailed cost of production of tuberlets using RMT is shown in Table 6. It is feasible to produce the seed requirement for 0.14 ha. with only Rs. 5000/=, thereby effecting a saving of Rs. 11,800/= to Rs. 17,400/= for every 0.14 ha. This method is

therefore very useful for the small farmer who cannot afford to purchase seed for his cultivation and to encourage farmers to produce good quality seeds for their own cultivation.

Table 6: Cost of production of "Tuberlets" using rooted stem cuttings in farmer's fields.

Cost of 2000 rooted stem cuttings @ Rs. 2/=	= Rs. 4000.00
Cost of field management for 40 m^2	= Rs. 1000.00
Total cost	= Rs. 5000.00
Cost of production of 10,000 tuberlets	= Rs. 5000.00
Area that can be planted with 10,000 tuberlets	= 0.14 ha.
Weight of "Seed tubers" required to plant 0.14 ha @ 2000 Kg/ha.	= 280 Kg
Cost of seed tubers @ Rs. 60-80 /Kg	= Rs. 16,800 - 22,400
Savings to the cultivator	= Rs. 11,800 - 17,400

Three other private cultivators in Boralanda (Badulla district) have set up their own net houses, and have started production of rooted stem cuttings for the production of seed potatoes on their own fields. The RARC, Bandarawela has supplied clean mother plants to all these cultivators. One of the cultivators has been able to produce over 6,000 rooted stem cuttings over a period of 5 months from 500 mother plants. These cuttings produced approximately 35,000 (475 Kg) Basic seed materials (G_1) in the open field. The farmer planted this volume of seeds in his paddy field and produced 7,250 Kg of consumption potatoes. Apart from rooted stem cuttings, the farmer had collected 3,000 pre basic seeds (G_0) from mother plants which subsequently produced 1,500 Kg of basic seed (G_1) seed upon multiplication. The other cultivator was able to produce 1,400 rooted stem cuttings from his net house. He planted these cuttings in a 32 m^2 field and produced 8,900 basic seeds (G_1).

The production and sale of rooted stem cuttings or basic seed is a highly remunerative enterprise, which can be taken up by unemployed youth and farmer organizations at village level.

Production of Basic Seed - 2 (G_2) in farmers field

Two farmers in Diyatalawa and Pitapola in Badulla District have planted Basic Seed - 1 (G_1) tubers, obtained from planting rooted stem cuttings in their highland fields during Yala 1992, to produce Basic Seed - 2 (G_2). Diyatalawa farmer who planted 200 Kg of G_1 seed produced 1800 Kg G_2 seeds and the other farmer who planted 170 Kg of G_1 seed produced 1900 Kg of G_2 seeds (Table 7).

Table 7: Production of basic seed-2 at farmers level.

Location	Cultivar	Basic seed-I (G_1)		Production of G_2 seeds (Kg)
		No. of tubers	Wt. (Kg)	
Diyatalawa	Sita	13,617	201	1800
Pitapola	Krushi	11,563	170	1900

This data indicates that by increasing the effective net house area in seed production farms and by construction net houses at village level, national seed potato requirements should be achievable.

In conclusion to improve the potato seed system the following points are stressed:

- Number of net houses in farms to be increased.
- Construction of net houses at village level to be encouraged.
- Rooted stem cuttings should be produced in the government seed farms from the mother plants obtained from RARC, Bandarawela.
- A quality control scheme should be developed.
- Efforts to control soil-borne diseases in the highland seed production fields should be intensified.
- Farmers should be trained in seed plot technique, to improve the quality of seeds that they produce.

Notes

Authors' address: P.W.S. Mallika Samarasinghe, Regional Agricultural Research Centre, Bandarawela.

Mechanical decapitation technique for rapid multiplication of pineapple planting material

H.M.S.Heenkenda and H. Samaratunga

INTRODUCTION

The demand for tropical fruits such as pineapple, mango, avocado, papaya and banana is continuously increasing in the world market. Pineapple exports from Sri Lanka earned about Rs. 4 million and Rs. 12 million in 1988 and 1989 respectively. Despite the growing potential in the world market, Sri Lanka is facing the problem of supplying large quantities of quality pineapple fruits regularly. There is insufficient supply for the local processing sector as well.

In 1991, the extent of pineapple cultivation in the country was about 3200 hectare. It is vital to expand this cultivation, but lack of healthy propagules is an impediment. There are four types of propagules used for pineapple cultivation, *viz:* crowns, slips, stem suckers and ratoon suckers (Fig.l). Since healthy propagules in large quantities are not available on time, the Department of Agriculture (DOA) has been trying to develop techniques for rapid multiplication of pineapple over the past two decades (Kotalawala 1971). Use of stem sections for multiplication, when introduced to the growers, failed to gain popularity because it involves intensive care. Also, casualties in the multiplication plots seem to be high and whole technique requires more than 7 months to produce an average size sucker, 400 - 600g in weight.

Eventhough, the tissue culture technique is very efficient for rapid multiplication, it has some shortfalls. Bartholomew and Criley (1983) discouraged tissue culture technique for mass propagation of pineapple because of the variability observed among the progeny. The suckers produced by tissue culture may cause several agronomic problems in the field (e.g. in fertilizer application, mulching, flower induction). Further it needs high skill and hence it is appropriate to develop alternate techniques.

MATERIALS AND METHODS

A new technique described as mechanical decapitation, has been developed for the rapid production of suckers. For this technique, mother plants have to be established in high density population. Suckers can be planted in furrows in the double row system, at a spacing of 100 x 30 x 30 cm to accommodate 51,000 suckers per hectare. The

density might even be further increased to give about 77,000 suckers per hectare or more. Suckers are maintained as usual according to DOA recommendations. When the plants have produced more than 15 mature leaves they are mechanically decapitated, using a device specially designed for this purpose (Fig.2). The decapitated plants are maintained as usual.

Fig. 1 Propagules of pineapple

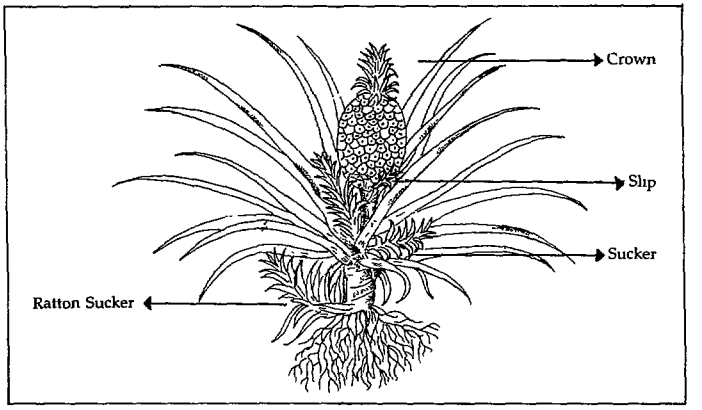
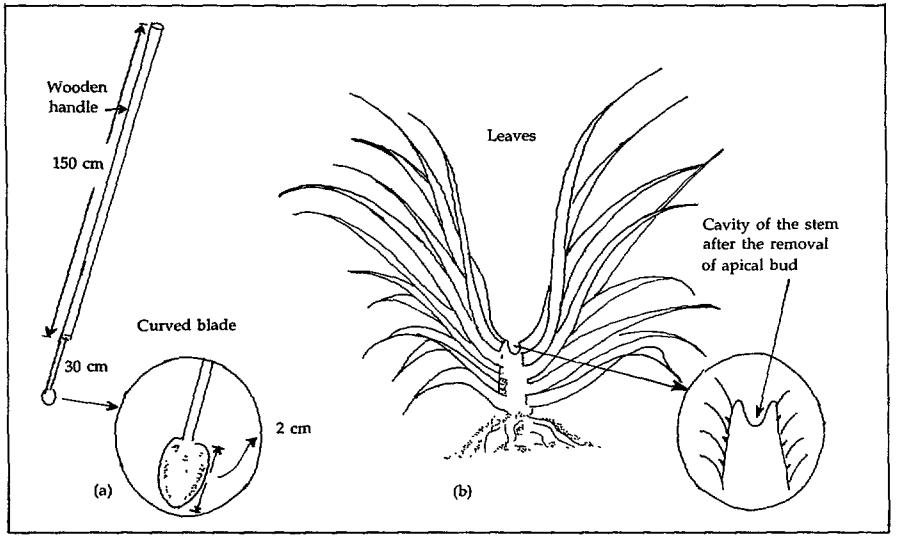


Fig. 2 Mechanical decapitation technique



a: instrument used for decapitation
b: pineapple plant after decapitation

RESULTS AND DISCUSSION

The plants produce suckers in their leaf axils, 4-5 weeks after decapitation (Fig. 3). These new suckers will grow for another 6-9 weeks to attain the size suitable for field planting (400 - 600g in weight). Average sucker production ranges from 4 to 8 per plant depending on the size of the mother plant. The larger the mother plant the higher the number of suckers produced (Fig. 4). The suckers can be harvested 9-12 weeks after decapitation (Fig. 5). After harvesting all the suckers, there will be another batch of suckers emerging which will attain the size suitable for field planting within another 8 to 10 weeks.

Fig. 3 Mean number of days for the first sucker to appear after mechanical decapitation of 'Mauritius' pineapple plants.

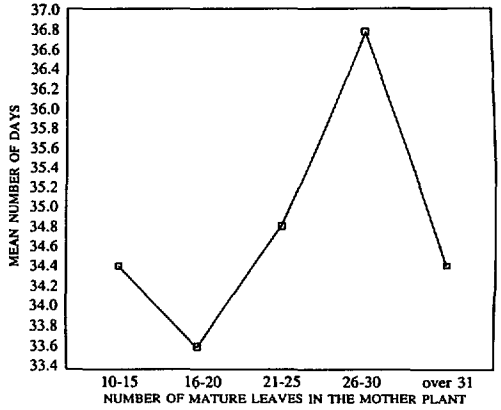


Fig. 4 Mean number of suckers produced by the plants of different size grops after mechanical decapitation of 'Mauritius' pineapple plants.

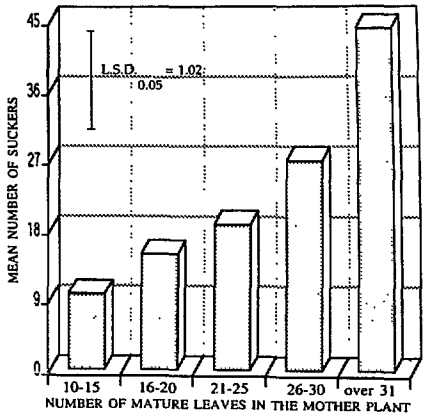


Fig. 5 Mean number of days taken to produce suckers of suitable size for field planting (400 - 600 g) after mechnical decapitation of 'Mauritius' pineapple plants.

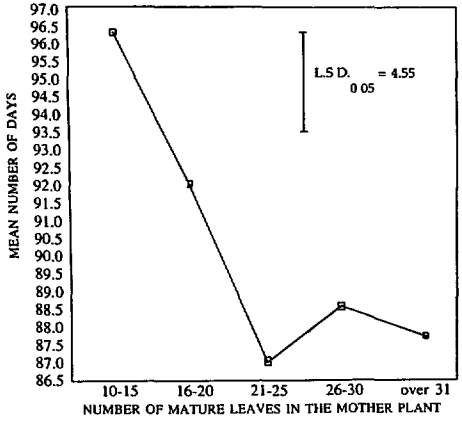


Table 1. Time from planting to fruit production

Propagule	Duration (Months)
Crown	>24
Slips	18-20
Ratoons	18
Stem sucker	12
Suckers from tissue culture	12-14
Suckers from decapitation	12-14

Table 2. Comparison of tissue culture and decapitation technique for sucker production.

Production Steps	Tissue Culture		Decapitation	
	Time taken (months)	Multiplication rate	Time taken (months)	Multiplication rate
1. Explant culture to 3rd proliferation	5	10 buds→10,000 plantlets	–	–
Establishment of mother plantation	–	–	8	750 mother plants
2. Proliferation to rooting	2	7500 plantlets *	–	–
1st cycle of suckers	–	–	3	4500 suckers
3. Plantlets in pots	2	7500 plantlets	–	–
2nd cycle of suckers	–	–	3	3000 suckers
4. Acclimatization	3	7500 plantlets	–	–
Total production	12	7500 plantlets	14	7500 suckers
Cost per sucker **		Rs. 3.00	1st cycle: Rs. 1.72 2nd cycle: Rs. 0.95	

* leaving an allowance for 25% contamination ** for a production volume of 1000 suckers

This cycle can be continued if one sucker per plant from the second batch is left to grow. Hence the decapitation technique offers a sort of a permanent nursery. The mechanical decapitation technique is comparable with other rapid multiplication techniques (Table 1 and 2).

CONCLUSIONS

Suckering can be promoted by mechanical decapitation in pineapple. Bigger the mother plant, higher the number of suckers produced and also the earlier the production. If sucker production is no longer required the plants could be induced to flower. Sucker production is more economical, simple and easy as compared with the other rapid multiplication techniques.

It is, therefore, suggested that pineapple growers could be encouraged to establish their own nurseries, using this technique to produce suckers to meet their own needs or towards self employment as suppliers of pineapple suckers.

References

Bartholomew, D.P., and R.A. Criley. 1983. Tropical fruit and beverage crops. *In*: Plant growth regulating chemicals. Vol. 2, Ed. L.G. Nickell. CRC Press, Boca Raton, Florida. 1-11 pp.

Heenkenda, H.M.S. 1992. Effect of plant size on sucker promotion in 'Mauritius' pineapple by mechanical decapitation. Unpublished/manuscript.

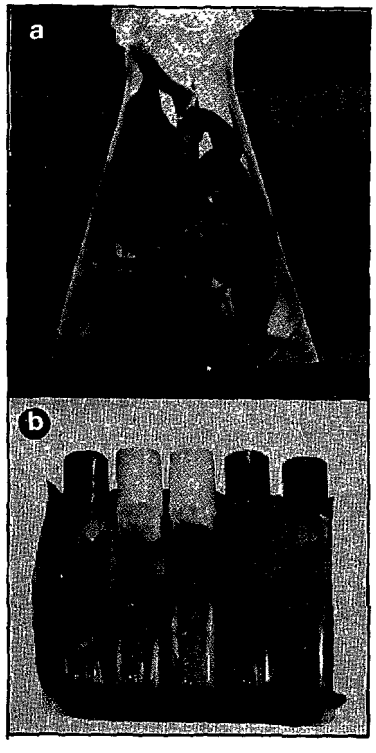
Kotalawala, J. 1971. Mass production of pineapple planting materials. *Tropical Agriculturist.* **127:** 199-201.

Sri Lanka Customs. 1989. Annual Reports. Colombo, Sri Lanka.

Wikramasinghe, A. 1992. Personal Communication.

Notes

Authors' address: H.M.S. Heenkenda and H. Samaratunga, Regional Agricultural Research Centre, Makandura.



Proliferation of cardamom shoots in liquid medium

Mass proliferation of calli derived cardamom plants

Plate 1. In vitro ***propagation of cardamom***

In vitro propagation of cardamom (*Elettaria cardamomum* Maton.)

D.B.R. Swarnatilaka

INTRODUCTION

Cardamom, popularly called "queen of the spices" is a dried fruit of *Elettaria cardamomum* Maton., a perennial herbaceous plant with irregular shaped rhizomes, belonging to the family *Zingiberaceae*. According to export figures, the average export earning of cardamom was 8.25 million rupees during the period of 1990-1991 and it was the largest foreign exchange earner among the spices in mid 1980s.

Cardamom is mostly cultivated on hilly areas, mainly in Kandy, Matale, Kegalle and Nuwara Eliya districts. Recently, area above 3,500 feet which are suitable for cardamom cultivation has been banded and reallocated for forest conservation by the Government. In the recent past, the area under cardamom cultivation has decreased considerably and the volume produced is below the export demand. In order to overcome this problem of land limitation, the Department of Export Agriculture is now carrying out a research programme to select suitable cardamom cultivars of high yielding ability with improved quality characteristics for low elevation areas.

Cardamom is propagated mostly by suckers and sometimes through seeds. However, seed propagation has many disadvantages. Plants produced from seeds show heterogeneity because of cross pollination. On the other hand single shoot of cardamom in the field produces only limited number of suckers. Also farmers are reluctant to remove suckers from the high yielding clumps. Therefore, this method of vegetative propagation by removing suckers does not meet the demand for the quality planting material. In addition, this vegetative propagation method carries over various pests and diseases like stem borer, nematodes, clump rot and viruses to the new plantations.

The *in vitro* propagation technique is now a commercially viable method to propagate many herbaceous plant species and it has great potential for application with the tropical plant species. A large number of uniform plants can be obtained in a comparatively short period of time from the selected or improved cultivars using this technique. Similarly, this technique could be used to develop new genetic variants through the mutation breeding programme. This will hasten dissemination of improved cultivars to the farmer.

Callus culture and meristematic or axillary bud culture are the two basic methods in *in vitro* propagation (Blake and Maxwell 1984). In callus culture, small pieces of tissue from any part of the plant is encouraged to de-differenciate into mass of cells which subsequently regenerates into the differentiated tissues. Finally, plantlets are generated through the process of organogenesis. Tissues from the active growing points treated with growth factors under precisely controlled conditions to obtain rapid growth and proliferation is the methodology followed in meristematic or axillary bud culture. Propagation through callus may result in variation amongst plantlets regenerated, and this can be overcome by using the organized tissues.

Cardamom can be propagated through *in vitro* technique. In 1962, Sirinivasa has reported the possibility of regeneration of cardamom plants through callus culture from seedling. Modified MS medium was introduced for cardamom by Yapabandara *et al.* (1989). Clonal propagation through *in vitro* technique was introduced for ginger by Hosok and Sagawa in 1977. Nadguda *et al.* (1977) reported that turmeric (*Curcuma longa*) can be regenerated using *in vitro* culture.

The recent trend in cardamom cultivation is to expand the area under cardamom cultivation. This can be achieved by introducing selected cultivar/s adapted to elevations where inter-cropping with rubber is possible.

The studies reported show that cardamom can be propagated using *in vitro* techniques. The modified MS media with the specific liquid-solid media transferring method showed a significant increase in getting good quality cardamom plants. Field establishment of these plantlets showed the similar growth pattern as the other field established cardamom plants.

MATERIALS AND METHODS

Newly emerged suckers of about 8-10cm long were used and washed with running tap water for about 10 minutes. After removing the outer leaves, these explants were dipped in 70% ethanol for 30 sec. and treated with teepol added 0.1% $HgCl_2$ for 20 minutes. After rinsing three times with sterilized distilled water, the final explants about 0.5cm were prepared by removing outermost layers. The explants were finally inoculated into the modified MS medium similar to the method followed by Yapabandara *et al.* (1989). All the cultures were maintained at 26°C temperature with the light intensity of 3000 lux having 16 hrs day length.

After period of two weeks, cultures in the establishment medium were transferred to the following media.

1. Solid modified MS medium for 12 weeks (solid – – ➔ solid).
2. Initially the solid modified MS medium for 6 weeks and later transferred into the liquid medium (solid – – ➔ liquid).
3. Initially liquid modified MS medium for 6 weeks and later transferred into solid medium (liquid – – ➔ solid).
4. Liquid modified MS medium for 12 weeks (liquid – – ➔ liquid).

Cultures on liquid media were kept on orbital shaker for slow agitation at 80 rpm. Following experiments were conducted using the propagated plant materials.

Determination of subculture frequency.

The cultured plants were introduced into the modified MS medium to determine subculture frequency. Data on shoot extension growth, discolouration of the medium and leaf colour were recorded.

Root induction.

Shoots of about 1-3 cm length were harvested from stock culture and introduced to the following media.

a. MS medium with IBA at 0, 0.2, 0.5, 1.0 and 2.0mg/1
b. MS medium with NAA at 0, 0.2, 0.5, 1.0 and 2.0mg/1

Rooting percentage and root length were recorded.

Evaluation of different potting mixtures

Plantlets obtained were transplanted in the following potting mixtures.

1. Soil: sand: cowdung, 1:1:1
2. Sand: coirdust, 1:2
3. Coirdust: sand, 1:2
4. Soil: sand, 1:1
5. Sand
6. Soil
7. Coirdust
8. Imported plug systems for tissue culture plants.

Percentage survival was recorded.

Evaluation of plants under the field condition.

In vitro produced plants were obtained and subjected to rooting and acclimatization process. After 3-4 weeks of acclimatization these plants were planted in potting mixture containing sand, coirdust (1:2). About 8-10 weeks later, the plants were established in the field at Kallebokka Estate under the *Eucalyptus* shade. Growth parameters of shoot extension, number of tillers and observations of flowering behaviour were obtained.

RESULTS

Explants when kept for six weeks in an agitated liquid medium and later transferred to similar solid medium for further six weeks showed the best performances in plant proliferation. The same treatment gave statistically significant higher shoot extension growth compared to the other treatments. The solid medium showed the lowest shoot extension (Table 1).

Table 1. Bud proliferation and shoot extension of the regenerated shoots after 12 weeks.

Media combination	No. of Buds	Shoot extension (cm)
1. Solid ------> Solid	0.57	3.15 [a]
2. Solid ------> liquid	0.57	4.46 [ab]
3. Liquid ----> solid	1.60	5.47 [b]
4. Liquid ----> liquid	0.68	4.79 [ab]
LSD	ns	1.63

* The value bearing the same letter indicates no significant difference at 5% probability level.

Although the liquid and solid treatments showed the highest number of buds compared to the other treatments, the results appeared not significant statistically. (Fig. 1). IBA appeared to be superior to NAA, and 2mg/1 of IBA gave 90% rooting with the highest root elongation after six weeks of introduction (Table 2).

The data collected on sub culture frequency showed that the highest rate of shoot extension (0.302 cm/week) was observed after four weeks (Table 3). Shoot extensions were measured at one week intervals.

Fig 1 Effect of different media combinations on the bud formation from the apical bud

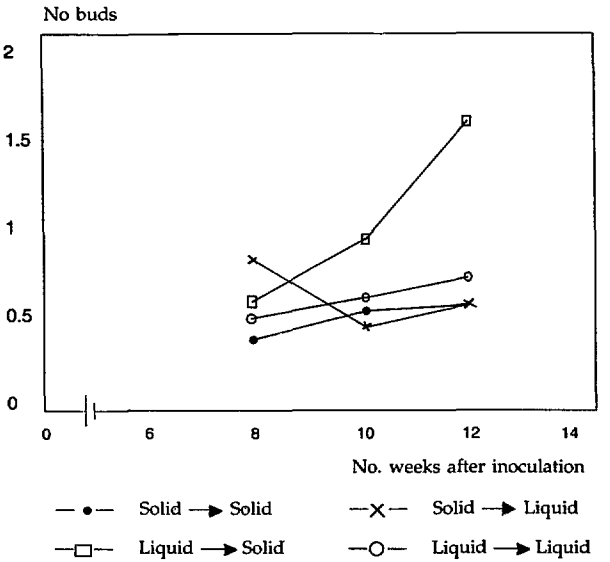


Table 2. Effect of different concentrations of NAA and IBA on rooting.

Growth regulator	Concentration (mg/1)	Percentage rooting (%)	Root length (cm)
IBA	0.0	02	0.02
	0.2	60	0.6
	0.5	80	1.2
	1.0	60	1.5
	2.0	90	2.1
NAA	0.0	–	–
	0.2	20	0.5
	0.5	40	0.9
	1.0	70	1.0
	2.0	80	1.0

Table 3. Determination of sub-culture frequency.

Duration (weeks)	Length (cm)	Incremental growth (cm)	Growth rate cm / week
0	2.231	–	–
1	2.278	0.047	0.047
2	2.432	0.201	0.154
3	2.683	0.452	0.257
4	2.985	0.654	0.302
5	3.106	0.875	0.121

The experiment conducted to evaluate different potting media showed that the mixture containing sand and coir dust of 1:2 ratio is giving about 50% survival compared to other combinations except the imported plug system for tissue cultured plants which gave about 90% of survival (Table 4).

Data showed no difference,between *in vitro* cultured and vegetatively propagated plants (Table 5). No statistically significant difference was found in number of panicles per clump, shoot height or tiller number after 30 months of field planting.

Table 4. Effect of different potting mixtures on survival of the *in vitro* produced plants.

	Potting mixture	Percentage survival
1	Soil: sand: cowdung 1:1:1	80
2	Sand: coirdust 1:2	50
3	Coirdust: sand 1:2	20
4	Soil : sand 1:1	10
5	Sand	3
6	Soil	–
7	Coirdust	40
8	Imported plug systems	90

Table 5. Growth performances of *in vitro* cultured plants and vegetatively propagated plants.

Treatment	No. of Tillers (cm)	Height of the tallest tiller per clump	No. of panicles
1. *In-vitro* produced plants	8.85	162.27	4.63
2. Vegetatively propagated plants	8. 32	156.24	4.23

DISCUSSION

The results of the experiments indicate that the modified MS medium in liquid followed by solid forms could produce good quality plant materials from the explants of cardamom (Plate 1). Explants of chinese tallow kept successively in solid and liquid MS medium showed the best performances in multiplication (Krikorian *et al.* 1987). The best subculture frequency obtained was four weeks. Most of the subcultures of nutmeg (*Myristica fragras*) at this stage have been transferred into the fresh medium after 4-6 weeks (Yapabandara *et al.* 1989).

MS medium supplemented with IBA (2mg/1) showed a remarkable superiority in rooting and root elongation. Fiorino *et al.*,(1983) have shown similar effects on root formation in shootlets of apple (*Molus domestica*) with IBA compared to NAA. IBA showed a significant effect on root formation in pear (Davidlane 1979).

The potting medium of sand and coir dust gave 50% survival of the tissue cultured plantlets and these plants established equally good as the plants which were vegetatively propagated using non-tissue culture technique in the field. Fiorio *et al.* (1983) have shown the best establishment of rooted plantlets of apple in sand and peat mixture. Better survival of papaya (*Carica papaya*) were obtained in potting mixture of soil, sand and vermiculite (Rajeevan *et al.* 1983).No significant difference was found among *in vitro* produced plants,suckers and seedlings of cardamom in the field (Lukose 1990).

It appears that the time taken for production of new plants which are suitable for field planting is about 20-24 months. The results obtained from the experiments suggest that *in vitro* culture of cardamom is a useful technique for rapid multiplication.

References

Blake, J., and P. Maxwell. 1984. Tissue culture propagation of Cacao by the use of axillary buds. *International conference on cocoa and coconuts.*

Davidlane, W. 1979. Regeneration of peas plants from shoot meristem tips. *Plant science letter.* **16:** 337-342.

Fiorino, P. and D.R. Leva. 1983. Propagation of Apple cultivar. *Acta Hortic.***131:** 95-99.

Hosok, T., and Y. Sagawa. 1977. Clonal propagation through tissue culture. *Hort. Sci.* **12(5):** 45-45.

Krikorian, A.D., and R.P.Kann. 1987. Aseptic manipulation of chinese tallow. *Cell and Tissue culture forestry.* **3:** 354-367.

Lukase, R. 1990. Annual report 1989-90, National Research Centre for Spices, India, pp.33.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. plant.* **15:** 473-497.

Nadgauda, R.S., A.P. Mascarenhas, R.R. Henore, and V. Jaganathan. 1977. Rapid multiplication of turmeric by plant tissue culture. *Indian J.Exp.Brol.* **16:** 120-122.

Nadgauda, R.S., A.P. Mascarenhas, and K.J. Madhusoodanan, 1983. Clonal multiplication of cardamom by tissue culture. *J.Plantation crops.* **11(1):** 60-64.

Rajeevan, M.S., and R.M. Pandey. 1983. Propagation of papaya through tissue culture. *Acta Hortic.* **131:** 131-139.

Yapabandara, Y.M. 1989. *In vitro* propagation of nutmeg and clove. M. phil thesis, Post Graduate Institute of Agriculture, University of Peradeniya, Sri Lanka.

Yapabandara,Y.M.,and J.Lekamge. 1989. *In vitro* propagation of cardamom. Research report for B.Sc (Agric). Faculty of Agriculture, University Peradeniya.

Notes

Authors' address: D.B.R. Swarnathilaka, Research division, Department of Export Agriculture, Matale.

Interest of the private sector in biotechnology and its achievements

C. Senanayake

INTRODUCTION

The aim of the private sector is to make use of the existing opportunities to produce goods and services required by the country. Experience the world over has shown that the private sector is a very effective agent of production to meet nearly all the material requirements of a society.

In Sri Lanka, agriculture has been the predominant form of production. From its inception in Sri Lanka, Ceylon Tobacco Company (CTC) has been primarily an agricultural producer. It is therefore natural for a company like CTC to introduce and develop the most recent technologies like biotechnology, specifically plant tissue culture, to meet both the company's and the country's needs and objectives.

The plant tissue culture laboratory at Kalagedihena was set up a little over a decade ago, initialy as a research and development laboratory to explore possibilities for commercial application of tissue culture to economically important plants. With its interest in commercial cut flower for export at the time, the company started on tissue culture of Orchids.

CTC'S present interest in biotechnology

Rapid clonal multiplication of ornamental plants for export and the local markets

In view of the demand for ornamental foliage plants internationally, the company embarked on a project to develop an export market for high value ornamentals. This was a major opportunity to bring in valuable foreign exchange to the country. Todate, ornamentals like syngonium, spathiphyllum, ficus, zantedeschia and ferns have been produced by tissue culture and exported to Europe, Japan and Australia. Tissue culture systems have now been successfully developed for species of *Dracaena, Cordyline, Anthurium andreanum, Pleomele and Aglaonema,* the latter two systems being developed by original research. Flowering was tested with anthurium and its commercial production commenced. Novel foliage plant types have been created by mutation breeding. Lately, the company has been thinking of ways to maximise uitlization of the laboratory, since

it is unique in being the only large-scale commercial laboratory in Sri Lanka, with a production capacity of 1.6 million plants per year. For this year, we will be restricting ornamentals to 40% of our total expected production of one million plants.

Production of high quality disease free elite fruit planting material

The tissue culture laboratory is now being looked at as a service centre to produce plants for domestic use by the company, as well as for the broader needs of the agricultural sector. In this respect, we hope to produce high quality disease-free elite planting material of fruit crops to help in achieving national targets of fruit production. Tissue culturing was successful and fruiting tested with banana, strawberry and passion fruit, while field evaluations are under way for tissue cultured grapes and pineapple. Tissue cultures have also been established with papaya. Commercial quantities of strawberry plants have been exported to Holland. Tissue cultured banana gave better yields and more sucker formation compared with conventionally grown plants. The varieties tested were Ambul, Kolikuttu, Anamalu and Williams hybrid. As such, this year we hope to produce large numbers (a hundred thousand) of banana plantlets. Pineapple too have been established to serve as a source of mother plants for our pineapple programme. A proud achievement for us is the success we had in developing rapid *in vitro* propagation for passion fruit by original research, since this technology was not available.

This technology can be utilized in the future to produce planting material. While this approach is commercially attractive to us it can fulfil the needs of large-scale growers who need large quantities of planting material that should be genetically uniform.

Tissue culture and micro propagation systems for potato seed production

Considering the company's requirements, the tissue culture laboratory has been producing *in vitro* plants of disease-indexed potato, which serves as starter material for the seed potato farm at Ambewela. Preliminary experiments on *in vitro* micro-tuber production were also successful.

Future Developments

Our future developments will be directed towards genetic improvement of crops via plant tissue culture for development of drought and disease tolerant varieties, improving the nutritional quality of certain crops and production of useful hybrid varieties of plants.

Notes

Authors' address: C. Senanayake, CTC Plantec Laboratory, Kalagedihena.

DISCUSSION

Session II: Application of Technology

Question - Prof. H.P.M.Gunasena: It appears from the presentations that were made here that the private sector has progressed rapidly while the government departments are yet researching and perfecting technologies. Is there now a need to reassess the biotech research in this country?

Comment - Dr. S.L. Amarasiri: We will discuss this very important question in the group discussion scheduled for this afternoon, as this involves policy matters.

Q - Dr. M. Sikurajapathy: What are the needs of the private sector from the public sector?

A - Dr. C. Senanayake: In the private sector, we do not have sophisticated equipments to do novel research and so forth. So, if the public and private sector can collaborate and do some important work such as selection of mutants for disease resistance or herbicidal resistance or improve the quality of certain food croops like cereals, it would be of great advantage to the country.

Q - Dr. M. Dassanayake: What is the relevance of tissue culture in the elimination of papaya and passion fruit viruses which are not seed-borne?

A - Dr. C. Senanayke: The advantage in tissue culture is that you can have a uniform crop and you can rapidly multiply superior plants free of disease.

Q - Dr. M.P. Dhanapala: Are the mutants shown by you in the ornamental plants from somaclonal variation or by mutagenic treatment?

A - Dr. C. Senanayake: They are not by somaclonal variation, but I have used gamma radiation to create those mutants. They are stable and true to type. I am doing shoot tip culture again with these mutants and the characters are stable.

Q - Mr. S. Amma: How do you detect potato virus? Do you have the rapid virus detection, Elisa method?

A - Ms. M. Samarasinghe: Yes. We are using the Elisa method to detect virus first in *in vitro* plants. Disease-free plants are used for further multiplication.

Q - Prof. H.P.M. Gunasena: The economics that you have worked out for rapid multiplication technology in potato are from simple laboratory experiments and so on. Now have you done anything really on a large scale to find out whether these figures are realistic to farmers?

A - Ms. M. Samarasinghe: For the production of mother plants, we have included the cost of chemicals, wages for the laboratory assistants, etc. We can produce mother plants at the rate of Rs.3/-. We have included the cost of constructing net houses and considered the production for 3-6 months from these mother plants both at farmer's level and at research station level. We have three successful farmers with net houses and cost was calculated according to their figures.

Q - Dr. Krishnamurthy: To what extent are farmers adopting the rapid multiplication technology in potato?

A - Ms. M. Samarasinghe: We have tested this scheme only for the last three seasons. On an average, and in one season about 10 farmers have practised this technology. Specially in Badulla district, farmers produce 90% of their own seed requirement. Technology has already gone to the farmers, but still this is at the demonstration level. Three farmers have already put up net-houses.

Q - Prof. H.P.M. Gunasena: Is there any country, where this technique of mechanical decapitation of pineapple is practised?

A - Mr. S. Heenkenda: No. It may be because in other countries other methods have been found satisfactory. In the Philippines, crowns and suckers are used. They regularly produce enough suckers under hygienic conditions and harvest suckers. They are gradually expanding their cultivation using their suckers. Some companies use the tissue culture technique with an intensive selection programme, but it increases the cost per plant.

Q - Mr. G.A. Gunatilake: Can't we use hormones to increase sucker production?

A - Mr. S. Heenkenda: There are some hormones which increase sucker production but they add to the cost. There are other problems too. Sometimes the leaves become very narrow when hormones are used.

Q - Can diseases be transmitted through suckers?

A - Mr. S. Heenkenda: Yes, there is a certain amount of risk involved. Also when the core is removed there is an open wound and decay can occur. However, in my experiments, I did not see any decay even during rainy weather. But we cannot rule out the disease problem. If there are any, they can be easily controlled.

Q - Mr. G.A. Gunatilake: You said, you are working with passion fruit and papaya. What steps do you take to eliminate virus in these plants? Virus is the most serious constraint in the cultivation of these crops.

A - Dr. C. Senanayake: In passion fruit, we start with a small piece of meristem of about 0.2mm to eliminate virus. With a large piece of meristem the chances of success are very high, but virus may not be eliminated. Additionally, it is better to give heat treatment as well.

Q - Mr. G.A. Gunatilake: Are your plants virus free?

A - Dr. S. Senanayake: Yes. Actually we have not started commercial production, but the technology is developed for future purposes.

Q - Dr. Panabokke: What is your selling price of banana suckers?

A - Dr. C. Senanayake: Rs.15/= a sucker. This year 100,000 plants and next year 200,000 plants will be available for sale.

Q - Are you selling pineapple plants?

A - Dr. C. Senanayake: We have produced 25,000 plants of Kew and 25,000 of Mauritius plants. Field trials are being conducted to study the fruit qualities. After these trials, the plants will be available for sale.

Q - Dr. Waidyanatha: Have you got any bearing papaya trees?

A - Dr. C. Senanayake: No, they are still in *in vitro* shoot multiplication stage.

Q - Dr. Dassanayake: Did the cardamom plants thrive under Eucalyptus?

A - Ms. Swarnalatha: The plants were obtained from Kelebokke estate where they were planted under eucalyptus. Therefore, I planted the *in vitro* plants also under eucalyptus to compare their performance. They were not affected by the eucalyptus trees.

Q - What was the spacing of eucalyptus plants?

A - Ms. Swarnalatha: 8 x 6 meters.

Overview

Session II: Application of Technology

First and foremost it is extremely important to identify the crops of national importance to invest in biotechnology. Secondly, there should be problems of national interest to work with in the selected crops.

Every year our country needs 18,000 tons of potato seed of which only half is met by local production. Potato is therefore a crop of national importance and the problem is supplying the demand for disease free planting material.

Pineapple, is also a crop of national importance. There is a demand for export and for local consumption. We have to supply the farmers with disease free planting material of varieties that are ideal for export markets. Consequently, there is a justification to use biotechnology to rapidly produce quality planting material.

Cardamon is an important spice crop in Sri Lanka. We were shown how tissue culture techniques can be used for rapid multiplication. There is a growing demand for foliage and ornamental plants for export. These plants are therefore, of national importance.

It is heartening to note that researchers have identified and are working on crops of national importance. Investments in biotechnology on these crops are therefore justified.

Scientists did not divulge the sources and methods used by them to obtain the latest information on biotechnology. Future biotechnology will involve not only tissue culture, but also gene transfer methods, gene sequencing, all types of DNA finger - printing etc., for which it is imperative to have latest information. Almost every week 50-60 research papers are published. It is therefore, necessary to have access to that data.

There are databases in biotechnology which we can have access to. In India, there is a database known as the "Nicknet" in which about 20 sub stations are connected to the central institute in Delhi. When a scientist wants to work on a particular subject in biotechnology he gets all the current information through computers and satellite from "Nicknet", some of which may not be available even in the latest journals.

The "Nicknet" has given a connection to Moratuwa University to work on Engineering subjects. If we get a connection to "Nicknet", our biotechnologists can work in parallel with other scientists in the world. Our aim is to solve national problems and therefore, we need to get current information. This should be done in collaboration with biotechnologists working on different crops . It is very important because the available knowledge can be pooled through collaborative research to have more success in biotechnology.

Such biotechnology results have to reach the farmers and we are happy to hear that already new technologies on potatoes, pineapples and ornamentals have reached the farmers.

I conclude by saying that, biotechnologists should not wait for seminars and workshop to get together. They should meet more often get into groups and collaborate and so that we will see successes very rapidly.

Dr. A.L.T. Perera
Discussant

SESSION III

RECOMMENDATIONS OF THE WORKING GROUPS

National policy and institutional mechanisms

The working group discussed the following five important issues.

- High tech that requires large investment in terms of physical and human resources.
- Modalities of technology transfer and associated problems including intellectual property rights.
- National-policy on biotechnology.
- Efficient integration of out-comes of biotechnology into existing agricultural practices.
- Identification of focal points for biotechnology development that can benefit agriculturists and assistance that can be rendered to such areas.

Considering the diversity of areas discussed, it was difficult to make specific recommendations in respect of many areas. However, group decided to make general recommendations that would be consistent with the objectives of this workshop.

RECOMMENDATIONS

Inventorisation of physical and human resources

The group realised that it is important to optimise the use of available physical and human resources. The laboratories are scattered throughout the country and there are very few researchers in the field of biotechnology. As a first step, therefore, it is essential to inventorise both physical and human resources available in the area of biotechnology. What is expected in the first instance, is to have a list of scientists who are working in biotechnology and inventorise the laboratory facilities.

Mechanism to facilitate inter-institutional collaboration

The importance of facilitating active collaboration among scientists working in different institutions to ensure proper integration of the research activities carried out in these institutions was recognised. It is, therefore, recommended that some mechanism be worked out to facilitate inter-institutional and inter-scientist collaboration.

Interface agency between researcher and farmer

It was clear that although there had been generation of technology by private and public institutions, the dissemination of information on such technology has been low. This is partly because there is no mechanism or agency to serve as an interface between the research organization and the farmers. Hence, there is a need to identify such an agency.

Complementing conventional technology with biotechnology

There was consensus that biotechnology cannot replace conventional techniques of crop improvement and production, and rather biotechnology should serve as an important complementary tool in crop improvement and production.

Recognition of scientists

In government institutions it is difficult to formalise property rights in respect of technology developed using public funds. Until modalities can be worked out scientists who make significant contributions should be recognised and adequately compensated. Presidential awards for outstanding scientists may be one method of recognition.

Inter-sector collaboration

As Sri Lanka is moving towards an open market economy where the private sector will play an important role in agricultural production, the need was stressed to have some kind of private sector collaboration, where appropriate, to facilitate research activities at state sponsored research institutions and also to harness innovative technology for increasing national productivity.

Supplementary research on rice biotechnology

It was pointed out that a considerable amount of work in biotechnology is being undertaken at IRRI. Much of this work is pertinent to countries like Sri Lanka and we can directly benefit from the results of their research. However, there are specific areas where the government institutions can undertake research on biotechnology in rice with expectations that such work will supplement the research at IRRI and other international organisations.

Dixon Nilaweera,	**H. P. Ariyaratne,**	**S. L. de Bogahawatta,**
Chairperson	Rapporteur	Facilitator

Current status, future needs and priorities

The working group identified eight broad areas for discussion and for organizing future biotechnology based research in the field of agriculture in Sri Lanka. These included the mature technological advances in different institutions; on-going biotechnological research; the focal points, future needs and priorities; plan of action; national and international co-operation and financial commitments.

Mature biotechnological advancements in different institutions

Application of biotechnology in the agricultural sector, tissue culture techniques in particular, is in progress both in the government and non government agricultural sector. Technology has been perfected for rapid multiplication of a some agricultural crops. The group identified the institutes that perform tissue culture based rapid multiplication of crops and the crops for which this technology has been perfected (Table 1).

Additionally, "Somaclonal variation", a phenomenon observed in regenerated plants from tissue culture, due to aneuploidy or other chromosomal aberration, is being utilized in the varietal improvement of sugarcane by the Sugarcane Research Institute (SRI) of Sri Lanka.

Biotechnological research in progress

Biotechnological research in progress comprises a list of broad activities performed in the DOA or elsewhere. For convenience of reference they are listed below:

- Micro propagation
- Germplasm conservation
- Germplasm exchange
- Embryo rescue
- Anther culture
- Meristem culture
- Cell and protoplast culture
- DNA transfer
- Mutagenesis
- Somaclonal variation

The research areas concerned are inter-related and are mostly based on cell and tissue culture techniques. Micro propagation techniques are used in the conservation and exchange of germplasm. Basic research conducted in these fields are also applicable in crop improvement work.

Table 1. The crops and the institutes conducting tissue culture based rapid multiplication programmes in Sri Lanka.

Crop	Institute	Remarks
Banana	DOA/CTC	I *
Potato	DOA/CTC	I *
Pineapple	DOA	II *
Mushroom	DOA/EAD	II *
Cardamoms	EAD	- -
Foliage	DOA/CTC	NS
Cut flower	DOA/CTC	NS
Strawberry	DOA	- -
Gloriosa	DOA	medicinal plant
Giant Bambo	IFS/DOA	
Forestry	FD	NS

* : Crop Research priority grouping by DOA
NS : Crop not specified
— : Not included in DOA research priority groups
DOA : Department of Agriculture
CTC : Ceylon Tobacco Company
EAD : Export Agriculture Department
IFS : Institute of Fundamental Studies
FD : Forest Department

Embryo rescue and anther culture research is directly applicable to plant breeding, particularly in self pollinated crops (eg.rice). Meristem culture is important in rapid propagation and in the elimination of viral infections from planting material. Cell and protoplast cultures are the basis for protoplast fusion (cybrids) and for gene transformation by non-vector mediated techniques (electroporation etc). Technology for subsequent whole plant regeneration is however needed if these techniques are to be useful. DNA transfer, is the ultimate tool of the breeder among the techniques available today for genetic improvement of plants. The success of recombinant DNA techniques however depends on many other unforeseen events such as gene expression, mutability, effects of background genome, integration of gene product into biochemical pathways of the transgenic organism and deleterious effects of gene product to human beings.

Mutations, as persistent alterations of the genetic make-up, are considered the only form of variability for crop improvement. Somaclonal variations described previously can also be considered a source of variability though sparingly utilized in crop improvement at present.

Focal points of bio-technology development

Three areas identified as focal points in biotechnology development are:

- Screening for stress factors *in vitro*,
- Single cell protein production,
- Fermented food processing technology,

Stress factors identified for *in vitro* screening at cellular level were both biotic (pest and diseases) and abiotic (salinity, alkalinity, herbicide tolerance, etc.). Single cell proteins, though not clearly defined, are useful gene products (alkaloids) that can be produced at cellular level. SRI is already planning for research activities in this field. Fermented food techniques involving microbial activities (soysauce, milk product, etc.) are also considered important focal points.

Future needs and priorities

The future needs and priorities were arrived considering the needs of rice improvement programme in Sri Lanka. With respect to other crops, same model can be followed where the areas of research are similar to that of rice. However, implications of using biotechnological procedures in the improvement of self pollinated crops vs cross pollinated crops should be well understood. Listed below are the future needs in order of priority.

- Screening for abiotic stresses (Fe toxicity, salinity etc.).
- Introgressing of useful alien genes by wide crossing and embryo rescue techniques.
- Exploiting somaclonal variants for genetic improvment.
- Shortening breeding cycle by dihaploid production from gametes of F_1 generation.
- Gene tagging (physiological traits - by RFLP) and screening segregants (cell or plants) at early stages.
- Recombinant DNA techniques to transfer alien DNA segments carrying desirable genes into rice plant.

Plans for future action

Each institution is expected to draw up future plan of action independently with respect to the crop and the area of research.

National and International co-operation

National and International co-operation is of paramount importance in the development of biotechnological research in Sri Lanka. Scientists who are actively engaged in biotechnological research should be identified to form a discipline - wise group. Group meetings should be held at least 3 times a year and a News Letter reporting advancements and progress made must be published. Joining regional and international biotechnology networks is recommended.

Management

Management is an important aspect in any research organization. An efficient system of research management should be adopted. Critical review of research projects periodically is suggested to improve the quality of research.

Finances

Biotechnological research is expensive and the researchers are advised to gauge the benefits before embarking on a research projects.

Prof. M. D. Dassanayake,	**M. P. Dhanapala,**	**S. Balendira,**
Chairperson	Rapporteur	Facilitator

Discussion

Recommendations of the working groups

Prof. Y.D.A. Senanayake: I would like to make some comments on the recommendations. First one of course is the recommendation that scientists involved in biotechnology research should meet periodically. There is also a request to inventorise the available physical and human resources in the country. Certainly these aspects are important and CARP can act as a facilitating organization. Second one is the need for a mechanism to facilitate collaboration and cooperation. The need is accepted. But which is organization that should be given the responsibility to facilitate? We need to identify the organisation. Then the question of recognition for the scientists in the field of biotechnology. In the developed countries because of the money involved, they do not simply stop at recognition. So simply having recognition in the form of presidential awards and so on will not help. To keep the best scientists working on biotechnology here, some kind of remunerative recommendation must be worked out. That could also be thought of by the facilitating organization which is given the responsibility of promoting collaboration among institutions.

In identifying the demands of the private sector on the public sector, we have to be very clear about what the public sector be doing and how far it should go about doing it and at what stage the technology be transferred to the private sector and the private sector moves in relation to the commercial aspects of that technology. Here again, there can be a reward mechanism built in for the scientists of the public sector, if suitable understanding and agreements are brought about between the two parties.

Dr. S.P.R. Weerasinghe: Thank you Prof. Senanayake. I would like to take up the points raised with regards to the recognition of scientists. It is a very volatile question. It is not only the scientists working in biotechnology who should be recognised, but all the scientists. As to whether it should be remunerative or just recognition, it is something which rests on a macro policy. I request the Secretary, Ministry of Agricultural Development and Research to give his views.

Mr. Dixon Nilaweera: The need for a mechanism to facilitate interinstitutional collaboration is recognised. There should be some system. But we cannot think in terms of an institution or organisation that will give directions. It will not work because there is no question of giving direction to any institution. The second issue is, now there are certain national priorities and there are certain imparts that are coming from the political system. There should be some agency by which all these are put together and a collaborative arrangement can be developed.

There are several institutions, but one which is already in existence is the Council for Agricultural Research Policy (CARP). The mandate of the CARP is specifically on agricultural research policy. While we develop the policy and send those messages down the system, the question is to what extent we could try to develop a system of collaboration. One particular idea that was suggested was whether we could think in terms of a subcommittee of the CARP which would try to get the various agencies to collaborate in terms of national policies. Now, there is another aspect that facilitates us to think in those terms. We have now decided to set up a subcommittee under CARP which will try to coordinate the agricultural extension systems. This committee is in place now. Therefore, of the several agencies and institutions which we possibly can recommend, one that is now in place is the CARP. With certain minor modifications, that institutional arrangement could be further strengthened to see to what extent this collaboration among various institutions could be brought about.

Dr. S.P.R. Weerasinghe: The question with regards to identifying the demand of private sector on public sector needs to be answered. We are looking at the private sector in a broader sense, not only the commercial private sector, the big people who are investing, but also the small investors who can take up these technologies. Because the thrust of the government is towards employment generation. There are lot of technically qualified people, may be they can take up this technology and start commercial enterprises. That is my perception. Here again the demand may vary from time to time, today it may be banana, tomorrow it may be pineapple and later it could be passion fruit and so on.

Mr. Dixon Nilaweera: Dr. Sikurajapathy asked the question, that while IRRI has developed so much of knowledge, should we really spend our money on doing research in rice? It is a basic issue and it was referred to our group for discussion. Then the question came, here is the case, where a technology has been developed and which may be applicable to our country. Should we just *per se* accept that or whether we should really look at it in practical terms and accept it. The question again raised was that some of the technologies developed in other countries may not be suitable to our country and probably it might bring along with it certain problems which may endanger our system. Therefore, in that perspective, we tried to find out to what extent the government could intervene in the whole system having discussed with the scientific community, what to be accepted. Actually, it is in the regulatory aspects that the state can intervene. Question was asked that if the private sector wants to get down some technology, is there any way in which government can intervene and stop it? It may not be possible but certainly, adequate safeguards must be developed to prohibit the wholesale import of certain technologies which may be of prejudicial interest to our country. It is in that context the state might have to play a role of intervention.

POSTERS

Elimination of virus in *Citrus* sp. through micro-grafting

D.G. Ranamukaarachchi, and K. Fernando

The feasibility of micro-grafting of 'Bibile Sweet' orange scion on to rootstock of woodapple and sour orange was studied. At the same time the effect of indoleacetic acid (IAA), benzylaminopurine (BAP), rootstock and physical state of medium on success of micro-grafting of citrus species was studied.

(l) Sour orange as rootstock gave better results than woodapple for 'Bibile Sweet' orange. (2) BAP concentration at 1 mg/l and IAA at 1 mg/l gave best results on both sour orange and woodapple rootstock. Hormone concentrations lower than the above adversely affected the success. (3) The highest grafting success was found when the medium was solidified with agar.

Notes

Authors' addresses: D.G. Ranamukaarachchi, Royal Botanical Gardens, Peradeniya; K. Fernando, Central Agricultural Research Institute Gannoruwa, Peradeniya.

In vitro propagation of Bamboo *(Thyrsostachys oliveri* Gamble) and Brazil nut (*Bertholletia excelsa* Bonpl.)

W.M. Abeyratne, and A. Yogarajah

Stem bud sections of bamboo (*Thyrsostachy oliveri*) were collected from field growing trees and cultured on modified MS medium for bud culture establishment and shoot multiplication. After 20 days in culture the explants produced axillary buds (1-3) in the medium supplemented with 2 mgl^{-1} BAP and 1 mgl^{-1} IAA. The same medium was also preferred for shoot multiplication. Shoots were successfully rooted in the MS medium modified with 3 mgl^{-1} IBA and 1 mgl^{-1} IAA. More than 75% of the rooted plantlets could be established in soil and later in the field.

Nucellar tissues of Brazil nuts were excised from sterilized seeds and cultured in modified MS medium for shoot regeneration. Maximum production of shoots (2-3), occurred in a medium supplemented with 2.0 mgl^{-1} BAP and 0.5 mgl^{-1} NAA: The shoots produced roots when transferred to MS medium modified with 1.0 mgl^{-1} IAA. The rooted plantlets could be successfully established in soil.

Notes

Authors' address: W.M. Abeyratne, and A. Yogarajah, Royal Botanical Gardens, Peradeniya.

In vitro propagation of Nedun (*Pericopsis mooniana*)

W.M.Abeyratne, D.C.Bandara, Y.D.A.Senanayake, and D.B.Sumithraarachchi

Hypocotyl, cotyledon and shoot tip explants were excised from actively growing 15-20 day old Nedun (*pericopsis mooniana*) seedlings grown *in vitro*, and cultured on modified MS medium. Callus cultures were established from hypocotyl and cotyledon explants in MS medium supplemented with 2.0 mgl^{-1} BAP and 5.0 mgl^{-1} NAA. Shoot buds occurred only in cotyledon callus. Hypocotyl callus failed to produce shoot buds in other combinations tested. Highest response in shoot bud organogenesis was observed in the medium supplemented with 5.0 mgl^{-1} BAP and 100 mgl^{-1} casein hydrolysate, in which over 60% of the cultures produced shoots.

Shoot tip cultures were established in the medium supplemented with different combinations of cytokinin (BAP or KN) and NAA. The highest response in culture establishment occurred in the medium supplemented with 2.0 mgl^{-1} BAP. BAP was a more effective promoter of multiple shoot proliferation than KN. Maximum proliferation of shoots occurred in a medium supplemented with 2.0 mgl^{-1} BAP. However, maximum production of growing shoots (5mm and longer) occurred in a medium supplemented with 2.0 mgl^{-1} BAP with 0.1 or 0.5 mgl^{-1} NAA. Shoots were successfully rooted in the medium supplemented with 5.0 mgl^{-1} IBA and 5.0 mgl^{-1} NAA, in which over 70% of shoots produced roots after 60 days in culture.

Notes

Authors' addresses: W.M. Abeyratne, D.C. Bandara, Y.D.A. Senanayake, Post Graduate Institute of Agriculture, Peradeniya; D.B. Sumithraarachchi, Royal Botanical Gardens, Peradeniya.

Meristem culture of Komarika (*Aloe vera* (L.) Burm. f.)

A. Hettiarachchi, D.P. Rajapakse, M.H. Mendis, P. Ganashan, and S.D.G. Jayawardene.

Komarika, a medicinal plant growing wild in Sri Lanka, is of commercial value and a potential export crop. It is used in Sri Lanka both internally and externally as medicines, and in Europe in beauty therapy and for preparation of a vast array of highly expensive body lotions. Commercial cultivation of this plant is hampered by lack of sufficient planting material. The plant does not produce seeds and is propagated by vegetative means. A protocol has been developed to propagate this plant using tissue culture technique. Cultures were established from shoot meristems in MS medium containing 3% sucrose, 0.02 mg/1 IBA, and 0.2 mg/l BAP. *In vitro* grown shoots, when transferred to media with high levels of BAP (5 - 10 mg/l), formed large number of shoots, about 70. The shoots formed roots upon transfer to soil.

Notes

Authors' address: A. Hettiarachchi, D.P. Rajapakse, M.H. Mendis, P. Ganashan and S.D.G. Jayawardene, Plant Genetic Resources Centre, Peradeniya.

Micropropagation of Niyangala (*Gloriosa superba* L.)

P.K.Samarajeewa, M.D.Dassanayake, A.Hettiarachchi, M.H.Mendis, and S.D.G.Jayawardena.

Niyangala, growing predominently in the shrub jungles of the wet zone of Sri Lanka, is well known for its extremely poisonous corms. Traditionally it has been used for treatment of several parasitic diseases and physiological disorders. The corm and the seeds of the plant contain several chemical compounds of medicinal value. There is a growing interest in large scale cultivation of this plant for the export of its seeds. The germinability of seeds is very poor and the use of the corm for multiplication does not provide enough planting material. Therefore a micropropagation technique for the multiplication of this plant was developed.

Shoot cultures were initially established in B_5 medium (solid) containing BAP (0.5-1 mg/l) and IBA (0.01-1 mg/l). Shoot multiplication occurred when shoots were transferred from B5 medium to MS medium (liquid) supplemented with BAP (1-5 mg/l) and IBA (0.5 - 1 mg/l). Root formation could be achieved when shoots were isolated and cultured in MS medium (solid) containing IAA (0.1 mg/l). These *in vitro* grown plants could be successfully established in soil.

Notes

Authors' address: K. Samarajeewa, M.D. Dassanayake, A. Hettiarachchi, M.H. Mendis and S.D.G. Jayawardena, Plant Genetic Resources Centre, Peradeniya.

Micropropagation and Somatic embryogenesis in Banana

H.P.V.Rupasinghe, M.H.Mendis, P.K.Samarajeewa, D.P.Rajapakse, and S.D.G.Jayawardene.

Conventional breeding, based on recombination and selection, is difficult to apply to a crop like banana as polyploidy and sterility are both serious handicaps to genetic improvement. Plant regenerated from tissue culture are known to exhibit genetic variability (somaclonal variation). Callus and cell suspensions are therefore potential tools for banana and plantain improvement. Studies were conducted to develop culture media for somatic embryogenesis in tissue cultures of banana and plantain.

Leaf base tissue were found to be better than meristem tissues for establishment of callus in modified Schenk and Hildebrandt (1972) medium. Tissues of Sinikehel (ABB) and IC_2 cultivar (tetraploid, AAAA) grew best in medium supplemented with 0.25 mg/l of 2,4-D, while Binkehel (AAA) and Kolikuttu (AAB) required 0.5 and 1.0 mg/l of 2,4-D, respectively. Rathambala (AAA), Muwanethikehel (AAB) and Alukehel (ABB) tissues grew well in the presence of both 2,4-D (0.25 mg/l) and BAP (1 mg/l). Somatic embryogenesis was observed in callus cultures of Binkehel, IC_2 cultivar, Muwanathikehel and Alukehel. Embryogenic calli, agitated in liquid MS medium (half strength) with BAP and 2,4-D (0.5 mg/l), produced cell suspensions with a large number of free floating embryoids.

Notes

Authors' address: H.P.V. Rupasinghe, M.H. Mendis, P.K. Samarajeewa, D.P. Rajapakse, and S.D.G. Jayawardene, Plant Genetic Resources Centre, Peradeniya.

Mass propagation of Anthurium (*Anthurium andraeanum* Linden ex Andre) *in vitro*

W.M. Abeyratne, and A. Yogarajah.

In vitro propagation of *Anthurium andraeanum* (red and orange) through callus culture was studied using immature embryos as starter explants. Callus induction and multiplication were favoured under low concentrations of auxin 2, 4-D while high concentrations of 2, 4-D were seen to be detrimental to the explants. Adventitious shoot formation was induced by transferring the callus cultures from dark to light conditions. Root induction and growth were favoured by IBA and successful establishment of plantlets in soil could be achieved under high humidity conditions.

Notes

Authors' address: W.M. Abeyratne, and A. Yogarajah, Royal Botanical Gardens, Peradeniya.

ACRONYMS AND WORKSHOP PARTICIPANTS

Abbreviations and Acronyms

A

ABA = abscisic acid

B

BAP = benzylaminopurine

BMICH = Bandaranaike Memorial International Conference Hall

BPH = brown plant hopper

C

CARI = Central Agricultural Research Institute

CARP = Council for Agricultural Research Policy

CISIR = Ceylon Institute for Scientific and Industrial Research

CRBS = Central Rice Breeding Station

CTC = Ceylon Tobacco Company

CW = Coconut water

D

2,4-D = 2,4 - dichlorophenoxy acetic acid

DA = Director of Agriculture

DARP = Diversified Agricultural Research Project

DNA = deoxyribonucleic acid

DOA = Department of Agriculture

E

E = embryonic

EAD = Export Agriculture Department

ELISA = enzyme-linked immunosorbent assay

F

FD = Forest Department

G

GA3 = gibberellic acid

GLH = green leaf hopper

I

IAA = indole - 3 acetic acid

IBA = indole butyric acid

IFS = Institute of Fundamental Studies

IRRI = International Rice Research Institute

J

JICA = Japan International Co-operation Agency

K

KN = kinetin

M

MAD&R = Ministry of Agricultural Development & Research

MS = Murashige and Skoog's basal medium

N

NAA = Naphthalene acetic acid

NE = nonembryonic

P

PGRC = Plant Genetic Resources Centre

PVP = polyvinyl poly pyrrolidone

R

RARC = Regional Agricultural Research Centre

r-DNA = recombinant DNA

RFLP = restriction fragment length polymorphism

S

SRI = Sugar Cane Research Institute

U

USAID = United States Agency for International Development

W

WBPH = White backed plant hopper

Workshop Participants

R.S. Abeysekera,
Department of Agriculture
(Provincial),
Matara.

W.M. Abeyratne,
Post Graduate Institute
of Agriculture,
Peradeniya.

P. Abeysinghe,
Agricultural Development
Authority,
Colombo.

S. Abeywickrama,
Regional Agricultural
Research Centre,
Ankunukolapelessa.

M. Agalawatta,
Division of Agricultural Economics,
Department of Agriculture,
Peradeniya.

P. Alahakoon,
Central Agricultural
Research Institute,
Peradeniya.

Gary Alex,
Project Officer (DARP),
USAID,
Colombo.

S.L. Amarasiri,
Deputy Director Agriculture
Research,
Research Division,
Department of Agriculture,
Peradeniya.

S. Amma,
Plant Genetic Resources Centre,
P.O. Box 59,
Peradeniya.

Glenn Anders,
Agriculture Development Officer,
USAID,
Colombo.

H.P. Ariyaratne,
Plant Genetic Resources Centre,
P.O. Box 59,
Peradeniya.

P.V. Arulpragasm,
Tea Research Institute,
Talawakelle.

S. Balendira,
Plant Genetic Resources Centre,
P.O. Box 59,
Peradeniya.

D.T. de S.L. Bogahawatte,
Technology Transfer Division,
Department of Agriculture,
Peradeniya.

E.M. Dassanayake,
Regional Agricultural
Research Centre,
Bombuwela.

Prof. M.D. Dassanayake,
Plant Genetic Resources Centre,
P.O. Box 59,
Peradeniya.

M.G. Dhanapala,
Agriculture Research Centre,
Girandurukotte.

M.P. Dhanapala,
Deputy Director,
Central Rice Breeding Station,
Batalagoda,
Ibbagamuwa.

Hon. R.M. Dharmadasa Banda,
Minister of Agricultural
Development and Research,
Ministry of Agricultural
Development and Research,
Getambe, Peradeniya.

Prof. C.B. Dissanayake,
Institute of Fundamental Studies,
Hantane, Kandy.

L. Dissanayake,
Central Agricutural
Research Institute,
Gannoruwa, Peradeniya.

G.A.M.S. Emitiyagoda,
Technology Transfer Division,
Department of Agriculture,
Peradeniya.

Iwasaki,
Japan International
Co-operation Agency (JICA),
Colombo.

H. Gamage,
Addl. Deputy Director,
Technology Transfer Division,
Department of Agriculture,
Peradeniya.

P. Ganashan,
Head,
Plant Genetic Resources Centre,
P.O. Box 59,
Peradeniya.

Prof. H.P.M. Gunasena,
Dean,
Faculty of Agriculture,
University of Peradeniya.
Peradeniya.

G.A. Gunatileka,
Deputy Director,
Regional Agricultural
Research Centre,
Bombuwela.

H.M.S. Heenkenda,
Regional Agricultural
Research Centre,
Bandarawela.

A. Herath,
Managing Director,
Mahaweli Economic Agency,
493, T.B. Jaya Mawatha,
Colombo 10.

L.G. Herath,
Deputy Director,
Regional Agricultural Research Centre
Bandarawela.

A. Hettiarachchi,
Plant Genetic'Resources Centre,
P.O. Box 59
Peradeniya.

K. Hirimburegama,
Faculty of Biological Sciences,
University of Colombo,
P.O. Box-1490,
Colombo 03.

W. K. Hirimburegama,
Faculty of Biological Sciences,
University of Colombo,
P.0. Box 1490,
Colombo 03.

A.R.P. Jayasinghe,
Technology Transfer Division,
Department of Agriculture,
Peradeniya.

H. Jayasinghe,
Director (Projects)
Ministry of Agricultural,
Development & Research,
Colombo 07.

K.D.S.M. Joseph,
Deputy Director
Regional Agriculture
Research Centre,
Maha Illuppallama.

S. Kathirgamathaiah,
Director,
Department of Export Agriculture,
Peradeniya.

M. Krishnamurthi,
Sugar cane Research Institute,
Udawalawe.

Prof. A. Kovoor,
Institute of Fundamental Studies,
Hantane, Kandy.

S.A. Krishnarajah,
Royal Botanical Gardens,
Peradeniya.

C. Kudagamage,
Central Agricultural Research
Institute, Gannoruwa,
Peradeniya.

A. Kuruppu,
Regional Agricultural Research
Centre,
Makandura.

A. Lekamwasam,
Deputy Director
Regional Agricultural
Research Centre,
Aralaganwila.

G.W. Liyanage,
Senior Agronomist,
Mahaweli Economic Agency
493, T.B. Jaya Mawatha,
Colombo 10.

K.N. Mankotte
Addl. Deputy Director
Technology Transfer Division,
Department of Agriculture,
Peradeniya.

M.H. Mendis
Plant Genetic Resources Centre,
P.O. Box 59,
Peradeniya .

S. Nagarajah,
Addl. Deputy Director,
Research Division
Department of Agriculture,
Peradeniya

Hon. H.G.P. Nelson,
State Minister of Agricultural
Development & Research,
Ministry of Agriculture,
Getambe, Peradeniya.

D.A. Nethsinghe,
Chairman
Central Atomic Energy Authority,
696 1/1, Galle Road,
Colombo 03.

D. Nilaweera,
Secretary,
Ministry of Agricultural
Development & Research,
Getambe, Peradeniya.

Asoka Nugawela,
Head, Plant Science Division,
Rubber Research Institute,
Agalawatte,

K. Ouchi,
Plant Genetic Resources Centre,
P.O. Box 59,
Peradeniya .

C.R. Panabokke,
Council for Agricultural Research Policy,
267, Galle Road,
Colombo 03.

Preston S. Pattie,
Chief of Party,
Diversified Agricultural Research Project (DARP),
Royal Botanical Gardens,
Peradeniya.

O.S. Peiris,
Chariman,
Sri Lanka Standards Institute,
P.O. Box 17,
53, Dharmapala Mawatha,
Colombo 03.

S. Peiris,
Department of Crop Science,
Faculty of Agriculture,
University of Peradeniya.
Peradeniya.

A.L.T. Perera,
Department of Crop Science,
Faculty of Agriculture,
University of Peradeniya,
Peradeniya.

B. Ragunathan,
Regional Agricultural Research Centre,
Makandura.

D.P. Rajapakse
Plant Genetic Resources Centre,
P.O. Box 59, Peradeniya.

R.G.A.S. Rajapakse,
Regional Agricultural Research Centre,
Maha Illuppallama.

S.M.S.D. Ramanayake,
Institute of Fundamental Studies,
Hantane, Kandy.

D. G. Ranamukaarachchi,
Royal Botanical Gardens,
Peradeniya.

T.T. Ranasinghe
Department of Agriculture (Provincial),
Colombo.

T. Sabaratnam,
Science Editor,
Ceylon Daily News,
Lake House,
D.R. Wijewardena Mawatha,
Colombo.

M.B. Samarakoon,
Technology Transfer Division,
Department of Agriculture,
Peradeniya.

M. Samarasinghe.
Regional Agricultural Research Centre,
Bandarawela.

S. Sathiyapalan,
Tea Research Institute,
Talawakelle.

N. Senanayake,
Deputy Director,
Regional Agricultural Research Centre,
Ankunkoloaelessa.

C. Senanayake,
Foliage Project,
Ceylon Tobacco Company Ltd.,
Kalagedihean.

Prof. Y.D.A. Senanayake,
Director,
Post Graduate Institute
of Agriculture,
Old Galaha Road,
Peradeniya.

G. Seneviratne,
Director,
Agricultural Research and
Training Institute,
114, Wijerama Mawatha,
Colombo 07.

Derrick Schokman,
33/4 Mission Lane,
Kotte.

V. Shanmuganathan,
Regional Agricultural
Research Centre,
Makandura.

M. Sikurajapathy,
Deputy Director Agriculture,
Seed Planting Materials Division,
Department of Agriculture,
Peradeniya.

G.A.C. de Silva,
Division of Agricultural
Economics and Projects,
Department of Agriculture,
Peradeniya.

T. Silva,
University of Colombo,
P.O. Box 1490,
Colombo 03.

Y. de Silva,
Central Agricultural
Research Institute,
Gannoruwa,
Peradeniya.

J.A. Sirisena,
Regional Agricultural
Research Institute,
Ankunukolapelessa.

A.D. Somapala,
Deputy Director,
Regional Agricultural
Research Institute,
Bombuwela.

H. Somapala,
Deputy Director,
Central Agricultural
Research Institute,
Gannoruwa,
Peradeniya.

J. de Soysa,
Central Agricultural
Research Institute,
Gannoruwa,
Peradeniya.

M. Subasinghe,
Regional Agricultural
Research Institute,
Makandura.

D.B. Sumithraarachchi,
Deputy Director,
Royal Botanical Gardens,
Peradeniya.

D.B.R. Swarnathilaka,
Department of Export Agriculture,
Matale.

T.M.A. Tennakoon,
Addl. Deputy Director,
Technology Transfer Division,
Department of Agriculture,
Peradeniya.

P. Vasudevah,
Technology Transfer Division,
Department of Agriculture,
Peradeniya.

N. Vignarajah,
Addl. Deputy Director,
Research Division,
Department of Agriculture,
Peradeniya.

U.P.S. de Waidyanatha,
Director,
Perennial Crops
Development Project,
Peradeniya.

S. Watanabe,
Leader, PGRC/JICA Project,
Plant Genetic Resources Centre,
P.O. Box 59, Peradeniya.

P. Weerakody,
Farm Broadcasting Service,
Department of Agriculture,
Narahenpita,
Colombo.

S.P.R. Weerasinghe,
Director of Agriculture,
Department of Agriculture,
Peradeniya.

T.G. Wettasinghe,
Executive Secretary,
Council for Agricultural,
Research Policy,
267, Galle Road,
Colombo 03.

A. Wickramasinghe,
Central Agricultural
Research Institute,
Gannoruwa,
Peradeniya

I.P. Wickramasinghe,
Department of Agricultural Biology,
University of Peradeniya,
Peradeniya.

W.M.R. Wickramasinghe,
Department of Export Agriculture,
Matale.

S. Wirasinghe,
Deputy Director Agriculture,
Technology Transfer Division,
Department of Agriculture,
Peradeniya.

N. Yogaratnam,
Deputy Director,
Rubber Research Institute,
Agalawatta.