NEW BIOTECHNOLOGIES

Proceedings of a Symposium

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COVER DRAWING

Artistic conception of the role of biotechnology in contributing to human welfare and progress.

Sun the provider of energy vital for survival of all life forms on earth. DNA, is the basic common molecular alphabet of all life forms - from microbes to man. What makes each organism unique or different is the making of the different chemical sentences i.e. the precise ordering of the chemical base units in a DNA molecule. The DNA double helix along with Sun brings forth this unity in diversity and the interdependance expressed among all life forms.

Biotechnology provides tools to change the chemical base units and modify life forms - Transgenic plants, animals, microbes. It opens the scientific horizon and promises better Food production, Healthcare and Human welfare.

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NEW BIOTECHNOLOGIES

Proceedings of the Biotechnology Symposium held on 14 June 1991 at ARTI Colombo

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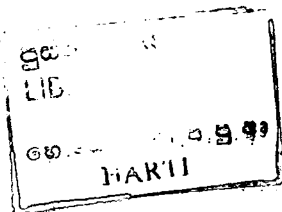

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PREFACE

Biotechnology is not new; in fact, it is as old as human civilization, if you consider that human civilization began at the time man first scratched the earth and planted seeds with the hope that he will harvest a crop he can dine on, rather than gather a handful of rice here, a corn cob there, a bamboo shoot from the other place and feed on them, on the hoof so to say. In its broadest sense, bio-technology refers to any technique whereby man adopts biology to his advantage or use or benefit. Therefore, if he uses a selected predator to control an insect pest, develops a special vaccine to control a dread disease (may be Malaria or Dengue fever) or uses tissue culture methods to produce large numbers of selected plants, we are using biotechnological techniques. However, in this country, the laymen seem to think that biotechnology refers only to tissue culture. This view was so widely held that, it was felt it would be an useful exercise to get several scientists, working on various projects involving biotechnology, to give a resume of their work to familiarize this work to the laymen here.

In the early inquires, we were not quite aware of the ramifications of biotechnology in this country and the varied specialized fields of its application, so we naturally concentrated on plant biotechnology, with the main emphasis on plant breeding and regeneration. We were then led to think that plant tissue culture methods were not yet developed to its fullest potential in this country. So we concluded that we will still have to import planting stock of very high-yielding varieties of crop plants, with special characteristics like the sweet taste and easy peeling qualities of certain varieties of **rambuttan,** or seedless guava, disease resistant **Hevea,** citrus and teak varieties from abroad. This brought in its wake the natural hazards of introducing virulent diseases of those and other plants, not already present in our country, from abroad. The cure was worse than the disease! So we had to think about the answer - plant quarantine, the prevention of the entry of new diseases into a country by precautionary measures backed by legislation. This was the original idea to link plant biotechnology to plant quarantine and say that, that was the state of the art here. However, as our inquires went on, we found that almost all the fields of biotechnology were covered by scientists in the local scene. We located talented persons working on such highly specialized fields as the technology of gene transfer, the production of vaccines against malaria (in fact there are three parallel studies being conducted on this very important and topical subject here), the use of gamma radiation in

plant breeding, and tissue culture was really a poor relation, in comparison. However, we kept a part of our original plan going, and held on to two interesting aspects of plant quarantine measures: first, the legislation and strict enforcement of the law on the one hand and the necessity to introduce large numbers of new varieties of plants on the other. The two may seem mutually exclusive at first look; but need not be so. We tried to highlight this.

The papers read covered the full scope of biotechnology and described work being done in Sri Lanka in the varied areas and aspects of the subject. It is a real pleasure to note that work is actually being done here on gene transfer, and the Medical College, Colombo, has to be congratulated on running a Master's programm in Bio-chemistry involving gene transfer.

Dr.O.S. Peries,
Chairman,
Sri Lanka Standards Institution.
July 1991.

FOREWORD

Biotechnology, in the sense of intervention in the evolution of "the natural order of things", has a very long history; like all the other living organisms, man too has affected the natural phenomena of which he is a part. The sense in which the term is used today, however, has its origins in the concept of man as being, in some sense, superior to the life around him and able to subject it, as a matter of right and without let or hindrance, to satisfy his own needs, wants, cravings.

'Man' is here used in the generic sense,but man and other living things inhabit and constitute a myriad different cultural settings. These cultural settings form the complex life support systems in which human culture values are shaped by the varying degrees in which they harmonise with the world within which they live.

The dominant accounts of "ecology", "environment", currently fashionable in the West, are largely self-serving and treat other natural phenomena from the perspective of the dominant segments of human society as things to be managed from afar. The selectivity shown in these accounts provides its own reading of what is being said. The impact of the new biotechnologies on the way of life of people in countries such as ours needs to be reviewed against this backdrop.

In Sri Lanka today, as elsewhere, the academic community lies marooned in little islets, little able to contribute to the human and intellectual leadership on which the survival of all societies depends and by which their character is determined. It is in this context that we invited one for most distinguished scientists, Dr. O.S. Peries, to organise this symposium. We are grateful to him for having done so and gratified that a continuing dialogue within our research community has been initiated through this symposium.

I thank the scientists who presented the papers which are reproduced here, the other distinguished participants who stimulated discussion at the symposium, Dr. Peries who put this publication together and Ms. Kala Maheswaran who assisted him in that task.

D.G.P. Seneviratne, Director,
ARTI.
July 1991.

Afterword

The demand for a reprint of this publication reflects the importance of the subject, not only for the research scientists who are engaged in studies in this field, but for the general public. It is a subject on which a number of perspectives exist, but are rarely articulated with equal force.

Among the perspectives that receive the tribute of being sought to be shut out in the media presentations that are engineered by the merchants of biotechnology, is that which relates to the immemorial knowledge that all living things possess, of the actions, which impact on each other and themselves all the time, that they must take in order to survive.

The dominant perspective, however, is that presented to a largely illiterate public in the industrialised societies through the television media; such educational programmes are, once more, only very rarely, "educational" in the sense that they seek to impart all information that is necessary for an understanding of the dimensions of the subject in their impact on the living world of human and others.

Bio-technology, so presented seeks to draw on the assonances that have been created in the sub-conscious and collective stock responses generated in industrial societies, in which the processes of growing up have come to be subsumed or even replaced by a thirst for extending their "life-span". Leonard Woolf, whom all Sri Lankans literate in either English or Sinhala know of, in his highly sympathetic and otherwise perceptive account of Empire and Commerce in Africa, published in 1922, referred to the African people as belonging to the "non-adult races". What the world is confronted with today, however, is a global challenge posed by the mind-bending and military power of the non-adult industrial societies.

Viewed in this context, the usefulness of developments in understanding biological processes would seem to be confounded by the uses to which such changes are sought to be put. The framework of the Dunkel Draft for GATT, which has in fact been far from a "general" agreement, presents all human kind in all parts of the globe, with an overt, no less, threat of what I would suggest is "biotech terrorism" on a scale that would make "narco terrorism" seem like a Sunday picnic. That picnic was a long time ago for most "developed" societies

while it still remains the central idiom in which the world view of "less developed" societies is expressed. The cynicism, self and all destroying, of the GATT "deal" seeks to put paid to all that. As one of the most perceptive commentators on industrial society, Naom Chomsky, recently put it (Scandinavian Journal of Development Studies, June-September 1993), "The cutting edge of industrial development is shifting to biology-based technology. That is one reason why the West, with the US in the lead, is insisting that GATT agreements and NAFTA (North American Free Trade Agreement) provide enhanced protection for patents ("intellectual property"), thus locking the Third World into dependency on high-priced products of Western agribusiness, biotechnology, the pharmaceutical industry, and so on. It is important to ensure that TNCs control seeds, plant varieties, drugs, and the means of life generally; by comparison, electronics deals with frills".

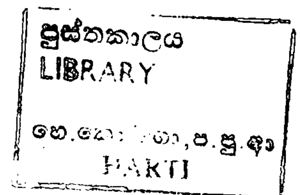
When Dr. O.S. Peries, whose "credentials" as an outstanding scientist need no accolade from me, died between the first publication of this book and the present re-print, the many tributes that appeared in the press referred to his character as a human being. His endeavours towards promoting studies in biotechnology among our research scientists may be said to have reached a point at which they could be informally formalised through the establishment of a biotechnology "centre without a centre" that I was privileged to have helped locate within the marvellous physical infrastructure of the ARTI.

I thank Dr. P. Ganashan, Director, Plant Genetic Resources Centre, Peradeniya for the trouble he has taken towards improving the text of the present presentation of that symposium. I have no doubt that all scientists whose papers are re-produced here and the many others who knew, loved, and respected him, would join me in dedicating this publication to the memory of Dr. Ossie Peries.

D.G.P. Seneviratne
Director/ARTI.

Contents

	Preface	iii
	Foreword	v
	Afterword	vii
1.	**Biotechnology in the year 2000** Ranjan Ramasamy	1
2.	**Gene Technology for Development** Eric Karunanayake	13
3.	**Research Towards Malaria Vaccine** Shiroma Handunnetti	35
4.	**Genetic Improvement through Induced Mutations** P. Ganashan	37
5.	**Tissue Culture of Tea** P. Arulpragasam	75
6.	**Screening of Yeast for Continuous Alcoholic Fermentation** U. Samarajeewa	83
7.	**Biotechnology for Plant Genetic Conservation and Breeding** P.K. Samarajeewa	89
8.	**The Contribution of the Private Sector to the Development of Biotechnology in Sri Lanka** S.B. Rajakaruna	101



9. Importing Planting Stock - The Importance of Plant Quarantine 107

S.N. de S. Seneviratne

10. The Needs of the Private Sector for New Plant Genotypes 114

R.L. de Silva

Appendix I - Seminar Programme 121

Appendix II - List of Participants 123

BIOTECHNOLOGY IN THE YEAR 2000

Ranjan Ramasamy, M.A (Cantab) Ph.D (Cantab)
Professor and Coordinator, Division of Life Sciences
Institute of Fundamental Studies, Kandy.

Introduction

It is sometimes useful, when discussing future trends in an area of technology, to consider the history of its development, since this gives the exercise a sense of perspective. Biotechnology, in its broadest sense, is as old as human civilization. Man has coexisted with his environment that contains other living organisms - bacteria, plants and animals, - since his beginnings. There has been an obligatory inter-dependence between man and the other forms of life eg. for food, and on the whole a balanced relationship has been established (at least until recently!). Occasionally in the past there have been traumatic imbalances eg. famine due to crop failure and epidemics caused by mutant infectious organisms. There has been a constant attempt by man throughout history to make use of and improve the terms of his relationship with the biological world. He has for example, developed better yielding, more drought resistant crops and utilised products of plants and lower animals to improve his quality of life eg. cotton, silk and antibiotics. The utilization of other living organisms for production geared to improving man's quality of life may be termed "Biotechnology." Taken in this sense, the practice of Biotechnology goes back a long way. Men in the Middle East are recorded to have transformed wild varieties of wheat into the cultivated varieties, through selection of desired traits, as early as 9000 B.C. Rice cultivation was reported in South East Asia by 4500 B.C. Domesticated varieties of sheep and goats were known by 7500 B.C. and cattle a few millennia later. The cultivation of cotton for producing cloth was recorded in the Indus Valley by 3000 B.C.

In the last decade however we have seen a very dramatic improvement in our understanding of the biological world, at the level of cells and molecules. Indeed the growth in this knowledge has been an exponential one and has been accompanied by a corresponding quantum leap in the practice of Biotechnology

The elucidation of the structure of deoxyribonucleic acid (DNA) in the early1950s was a landmark in both Chemistry and Biology and gave birth to a new science - Molecular Biology. This and the subsequent discovery of restriction enzymes i.e. enzymes that cleave DNA particular sequences of nucleotides, were critical scientific discoveries that led to the development of the technology of gene cloning. Modern biotechnology makes use of numerous such advances in basic or fundamental science to exploit the potential of other living organisms for human benefit. The definition of Biotechnology that I use in this lecture is somewhat broader than what is often stated elsewhere. For example the European Federation of Biotechnology in 1981 adopted the following definition: "Biotechnology is the integrated use of biochemistry, microbiology and chemical engineering in order to achieve the technological application of the capacities of microbes and cultured cells".

Different Areas of Biotechnology

Biotechnology may be broadly classified, for the purpose of this lecture, into three areas depending on the type of human activity involved. These are biotechnology in relation to Health, Agriculture (including Animal Husbandry) and Industries.

Biotechnology in Relation to Human Health

Biotechnology can be applied to improving the health of nations in many ways and I would like to illustrate a few of these in some detail.

(i) Development of Vaccines

Infections are a major cause of human illness and death in tropical countries. Poverty, undernutrition, illiteracy, lack of proper medical care and poor hygiene contribute to the spread of infectious organisms which include viruses, bacteria, protozoans and worms. Immunization remains the principle method of preventing infectious diseases. A notable success of the vaccination approach has been the eradication of smallpox. Vaccines based on defined molecules of the pathogens (termed molecular or sub-unit vaccines) afford many advantages over the more conventional vaccines based on attenuated or dead pathogens. Indeed in the case of a malaria vaccine, conventional vaccines are

unrealistic because *Plasmodium falciparum* is cultured in human blood and such blood-derived vaccines may transmit pathogens such as the human immunodeficiency (AIDS) and hepatitis viruses. Different forms of molecular vaccines are : a) a recombinant proteins based on the antigens of pathogens, cloned and expressed in bacteria, yeast or suitable eukaryotic cell lines, b) synthetic peptides containing the epitopes recognised by protective antibodies and immune effector cells and c) recombinant viral vectors (carriers) such as **Vaccinia** engineered to carry genes coding for pathogen antigens The production of molecular vaccines on a large scale, is an area of Biotechnology that has particular relevance to countries such as Sri Lanka. Such ventures are not unrealistic. For example, a recombinant vaccine against Hepatitis B, based on the major viral surface protein, is already marketted by Smith, Kline and French. My laboratory at the IFS is interested in the possibility of developing a synthetic vaccine against malaria. We have every reason to hope that we will have a malaria vaccine before the end of the decade.

(ii) Immunodiagnostics

The diagnosis of disease often depends on serological typing of pathogens. Polyclonal antibodies present in most typing sera often lack adequate specificity in this regard. The advent of monoclonal antibodies has revolutionised immuno-diagnostics. Because of their unique specificity, they can be useful in detecting minor variants of a pathogen eg. Dengue subtypes. To give you a practical example, let me cite an instance where, before commencing work on an Australian arbovirus in Brisbane, I got myself tested for previous exposure to the arboviruses. The test consisted of screening my serum against antigens from a panel of different arboviruses. To the great concern of the technician it appeared that I reacted positively with all of the half dozen or so virus antigens, some of which were derived from quite lethal viruses. The matter was cleared up only when I remembered that I had been vaccinated against Yellow fever, which is caused by an arbovirus, many years previously. Obviously my serum contained antibodies against the Yellow fever virus which cross-reacted with all the other arboviruses that were included in the test. Such a serum would therefore have been useless for specific diagnosis of an arbovirus infection. The reaction of a monoclonal antibody on the other hand can be made much more specific for a single arbovirus.

(iii) DNA Probes in Diagnosis

The enzyme DNA polymerase had the distinction of being described as the molecule of the year by the journal "Science" in 1989. This is because a heat stable form of the enzyme isolated from a bacterium that grows in hot water can be used in a process known as the Polymerase Chain Reaction to generate millions of copies of a given sequence from a molecule of DNA. The PCR reaction has spawned many applications and one of the most useful is in the diagnosis of disease. Where the DNA sequence of a part of the pathogen genome is known, the PCR reaction can be used to amplify the sequence over a millionfold, the amplified DNA can then be detected with radioactivity or enzymatically labelled and synthetically made complementary DNA, termed a DNA probe. The use of DNA probes provides a very sensitive and specific assay for the detection of early stages of infectious diseases.

(iv) Cancer Immunotherapy

In cancer therapeutics monoclonal antibodies are directed against tumour-specific antigens that are also covalently linked to a cell toxin molecule. Such antibodies have been termed "magic bullets" but their use in man is still held back through some practical problems. One problem is that many monoclonals are derived from mice and that patients mount an immune response against the foreign antibodies. However, it is now possible to make human nomoclonal antibodies and indeed genetically engineer mouse monoclonals to assume human antibody characteristics. Many of the problems associated with magic bullets are likely to be overcome with the decade.

(v) Cancer Imaging

Cancer imaging is carried out through tumour specific monoclonal antibodies that are tagged with radioactive or heavy atoms. This technique yields information on the location and spread of tumours by a relatively non-invasive technique, that would be hard to obtain otherwise.

(vi) Tissue Typing

Before transplantation it is necessary to match the donor and recipient tissues for histocompatibility antigens. This is to avoid graft rejection determined by incompatible antigens. Monoclonal antibodies, because of their great specificity are very valuable in this task. Similarly monoclonal antibodies are becoming the reagents of choice for blood group determination.

(vii) DNA Fingerprinting in Forensic Medicine

The pattern of restriction enzyme digested DNA after separation by size on an agarose gel is characteristic of an individual. The use of the PCR reaction improves the sensitivity of the detection which is performed with a radiolabelled or enzymatically labelled DNA probe. This technique can be used to trace the origin of human tissue samples such as blood (2-5 microlitres), hair follicles (single hair) and semen (1-2 microlitres). Forensic evidence of this nature is now accepted in many law courts and is likely to become widespread within the decade.

(viii) DNA Analysis

DNA analysis is used in determining genetic predisposition to inherited diseases. Considerable effort is being directed towards identifying the genes responsible for many inherited diseases. Genetically transmitted diseases include many types of cancer, haemoglobinopathies, cystic fibrosis, muscular dystrophy etc. While many of the responsible genes have been identified, it is possible to associate several unidentified genes with specific changes in restriction enzyme digest patterns termed Restriction Fragment Length Polymorphism (RFLP). The RFLP patterns can then be used in diagnosis. This analysis can be performed on DNA, isolated from the cells in the amniotic fluid and provides a method of antenatal diagnosis. An analysis of the DNA of prospective parents, with known disorders in the family, provides a basis for genetic counselling. Kits for DNA analysis for specific inherited diseases are already in the market and will become more common in the years to come.

(ix) Production of Physiologically Active Proteins

Many proteins that are pharmacologically active or useful have the potential for production by recombinant DNA techniques in commercially viable quantities. Molecules such as tissue plasminogen activator and interferon are already produced in this way. We can anticipate many more recombinant proteins, particularly blood products, to become available soon.

(x) Gene Therapy

Gene theraphy i.e. the correction of genetic defects and the improvement of humans by the introduction of new genes at the somatic stage, is the ultimate dream of molecular biologists. As a matter of fact, this dream became a reality in a small way in May 1989 at the National Institute of Health in Betheda, Maryland, USA. A team of investigators introduced the gene for neomycin resistance into tumour infiltrating lymphocytes (TIL) from a patient terminally ill with melanoma. The TIL treated with IL-2 treated recombinant TIL were reintroduced into the patient in order to follow the tissue localisation of the introduced cells by the use of the neomycin resistance marker.

In the near future experiments with recombinant stem cells carrying the gene for adenosine deaminase, which affects lymphocyte function and causes immunodeficiency are planned. While gene therapy will undoubtedly be practised in the future, it is likely to be limited to a few diseases and will be restricted by ethical considerations.

Biotechnology in Relation to Agriculture and Veterinary Medicine

Many of the applications of Biotechnology that have been described for human medicine are also of value in Veterinary medicine eg. diagnosis of infections, vaccines, etc. However, a major application of genetic engineering in regard to animals is in stock improvement. The production of transgenic mice is now a relatively trivial procedure and involves the introduction **in vitro** of embryo cells from one strain of mice into the blastocyst of another, followed by implantation into a pseudo-pregnant female mouse. The donor cells, which can of course be from the same strain as the host, may be engineered to carry specific genes and if they are puripotent stem cells, the latter may be able to transmit the introduced gene through the germ line.

The introduction of human growth hormone gene into mice in this manner leads to gigantic mice, because of overproduction of the hormone. However the same gene in pigs does not lead to a much bigger animal, presumably because selective breeding by man has already ensured an optimal size. But there are numerous physiological changes both favourable (production of leaner meat) and unfavourable (physiological disease). These results are only

a beginning and other work in progress include, for example, the production of sheep with improved wool quality. Genetic engineering is likely to go hand in hand with traditional breeding procedures for stock improvement in the future.

It has been estimated that Plant Biotechnology is a hundred times bigger than human health related Biotechnology in terms of the potential financial turnover. Much of the activity in Plant Biotechnology involves the use of tissue culture for the propagation of elite genotypes and the use of genetic engineering to produce plants of the desired genotype.

As a simple example of the use of plant tissue culture, we can take the case of the giant bamboo. It has been suggested that soil erosion along the banks of the Mahaweli river may be minimised by planting the giant bamboo. The bamboo has an extensive root system that binds soil well and is also a plant that grows well in the river banks. However, this plant only flowers once in every 70 years or so and therefore tissue propagation to obtain plantlets may be the only way of obtaining large numbers of plants of a specific type, free from viruses and other pathogens. Another application of tissue culture is as an additional effort, after the collection of seeds, to preserve plants in danger of extinction. Valuable germplasm that is fast disappearing may be preserved in this manner.

As an example of genetic engineering we may consider the production of herbicide resistant cereal plants, although the widespread use of herbicides is not recommendable due to adverse environmental consequences. It is possible to isolate a gene for herbicide resistance and recombine the gene into the plant genome thereby producing a transgenic plant resistant to specific herbicide. Fields of such herbicide resistant plants can then be aerially sprayed to remove weed growth hence improving yields relatively cheaply. Crop plants that are resistant to bromoxynil and atrazine have been produced in this way.

Genetic engineering in plants has involved the cloning of the gene of interest in an *E. coli* - *A.tumorfaciens* shuttle vector and then recombination into the Ti plasmid of *A.tumorfaciens*. This is in turn used to infect plant tissue and transfer the gene of interest into the plant genome by recombination. The *Agrobacterium* technique works well with tomato and potato plants of the family *Solanaceae* but the results with monocotyledonous cereals and other economically important plants has been unsatisfactory. Electroporation, where

genes of interest are directly introduced into the plant genome, through pores in the cell membrance induced by an electric current, is now the technique of choice for plant cells.

The production of genetically engineered plants has aroused concern among environmentalists. The concern being that, as a result of major gene polymorphisms, invasive weeds may be produced in genetically engineered plants. Also, engineered plants may be able to transfer their traits by normal sexual reproduction to neighbouring weedy relatives. In all these instances, invasive weeds can be the result (eg. carrying the gene for herbicide resistance). The solution to this dilemma lies, of course in careful regulation of the release of genetically engineered plants.

Similarly genetic engineering can be used to introduce many other desirable qualities eg. insect pest resistance, pathogen resistance, better seed quality etc. into target plants.

Other major applications of biotechnology in agriculture include:

(i) Identification of plant pests (especially in seeds) using DNA probes and monoclonal antibodies.

(ii) Production of improved nitrogen fixing symbionts eg. those that are better adapted to unfavourable soil and climatic conditions.

(iii) Use of RFLP[1] analysis to identify useful genes in the plant genome. This method can greatly simplify the process of classical plant breeding.

(iv) The production of genetically engineered microbes for the biological control of insect pests. The classic example here is *Bacillus thuringiensis*, which produces a delta endotoxin that kills many insect species. Insects however can develop resistance to the toxin. Genetic engineering may be usefully applied to produce toxins with greater

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1 RFLP Analysis - Restriction Fragment Length Polymorphism

specificity and to overcome insect resistance. A recent development lies in the production of juvenile hormone esterase by *Baculovirus*, a virus specific for lepidopteran insects. The enzyme breaks down juvenile hormone in insects which is necessary for the maintenance of larval stages. Normally the esterase makes its appearance at the late larval stage thereby reducing the juvenile hormone levels which in turn gives rise to pupation. If a *Baculovirus* containing cloned esterase is used to infect young larvae, the larvae stop feeding and die. Such a *Baculovirus* is another potential genetically engineered insecticide.

Industrial Biotechnology

The production of ethanol by yeast induced fermentation is an age old biotechnological process. However this has now been extended to the production of other industrially important chemicals such as acetic acid, amino acids, fructose, antibiotics etc. Enzymatic processes are being developed for the economical conversion of woody material and other agricultural by-products into useful substances such as sugars. In Sri Lanka, we have the potential to use tea waste and rice straw. Of course, plants may also be specially grown for providing material for bioconversion. It should be noted in this context, that plant cells in tissue culture are a source of medicinal chemicals, flavours and vitamins.

Enzymes are also beginning to find uses as probes to detect concentrations of chemicals eg. oxygen, in industrial manufacturing plants i.e. they act as biosensors. A simple example of this use is the dipstick method for detecting glucose in urine, for diagnosing diabetes, where the enzyme involved is glucose oxidase.

Major advances have also been made in the use of micro-organisms for the treatment of wastes. A bacterium that degrades hydrocarbons, and is therefore of use in clearing oil spills, created history by becoming the first living organism to be patented in the world. The treatment of sewage effluents and industrial effluents containing toxic chemicals, using micro-organisms, is an area of intense investigation.

Another useful application is the use of microbes in mineral extraction. As rich mines become exhausted, we find ourselves turning to leaner deposits of

metals. Microbial leaching provides the answer as it is cheaper, environmentally sound and capable of efficient extraction eg. the bacterium *Thiobacillus ferroxidans* has been used for extracting copper in India.

Enzymes are proteins and in nature, organisms have evolved enzymes that can perform a marvellous array of catalytic reactions. Like inorganic catalysts, enzymes function by reducing the activation energy of a chemical reaction. However enzymes, by virtue of their protein nature have a far greater substrate specificity than inorganic catalysts. If the chemists can harness this property by tailoring enzymes for specific reactions that do not occur in nature, a new era in synthetic chemistry will be opened.

Now immunologists have done just this with protein antibodies. Antibodies made to transition state analogues of a chemical reaction are able to function as catalysts in a variety of chemical reactions, some of considerable industrial importance. The rate of enhancements observed so far have been variable but are likely to be improved in the near future.

POTENTIAL ADVANTAGES OF AN INITIATIVE INTO BIOTECHNOLOGY IN THE COUNTRY

a. One of the major effects will be to put us back in line with many countries in the world vis-a-vis the expertise in Biotechnology. The stimulation of scientific expertise will have an impact on other aspects of the Sri Lankan economy as well as in improving academic standards in research and tertiary educational institutions.

b. Encouraging initiatives in applied Biotechnology projects will lead to significant improvements in the Industry, Health and Agriculture sectors.

c. Appropriate investment can lead to export oriented biotechnology industries eg. in fields such as medical diagnostics, tissue culture propagated plants, industrial chemicals from biomass and etc. These industries will rely on the relatively inexpensive but competent labour available in Sri Lanka.

d. Investments in biotechnology industries can also lead to significant import substitution over the next few decades, since there is likely to be an increasing demand in Sri Lanka for biotechnology products in the Health and Agriculture sectors.

e. Employment opportunities for qualified youth in biotechnology related activities is bound to increase. Although it is difficult to estimate numbers, a prediction of several tens of thousands for a relatively low investment may not be an unreasonable estimate.

CONCLUSION

It is reasonable to conclude therefore that modern Biotechnology offers exciting prospects for improving the quality of life of man, here in Sri Lanka and elsewhere in the world over the next decade. We must sow the seeds of Biotechnology immediately if we are to partake in the harvest to come.

GENE TECHNOLOGY FOR DEVELOPMENT

Professor Eric H. Karunanayake, MBBS, Ph.D.
Department of Biochemistry
University of Colombo, Sri Lanka

An explosion of knowledge is shaking the science of biology, an explosion that is already touching the life of each one of us. At its centre is chemical information - information that our cells use, store and pass onto subsequent generations. With this new knowledge comes the ability to restructure the molecules that program living cells. Already this new technology is being used to solve problems in diverse areas such as waste disposal, synthesis of drugs, treatment of cancer, plant breeding, diagnosis of human disease and most recently in forensic science.

The new biology also tells us how the chemicals in our bodies function; we may soon be programming ourselves and writing our own biological future. When this happens, each of us will be confronted with a new set of personal and political choices. Some of these difficult and controversial decisions are already upon us, and the choices will not get easier. Informed decisions require an understanding of molecular biology and recombinant DNA (rDNA) technology or more popularly known as Genetic Engineering.

Genetic engineering or rDNA could be defined as the manipulation of the information content (genetic material) of an organism to alter the characteristics of that organism. Genetic engineering may use simple methods like selective breeding or complicated ones like gene cloning. The use of such

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A lecture on the same topic was delivered by Prof. Karunanayake earlier.

This is reprinted from: The Proceedings of the First Annual Session of the Organization of Professional Associations of Sri Lanka, 6-9, October 1988.

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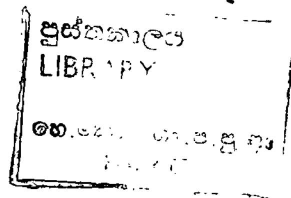

genetically engineered organisms for the large scale production of pharmaceuticals, hormones, agricultural products is a major aspect of present day biotechnology.

The chemical information which makes a microbe different from a whale or a man from a mouse is contained in Deoxyrybo-Nucleic Acid (DNA). The genetic engineering industry that is rapidly advancing today, owes its success to the famous discovery in 1953 by Watson and Crick, that DNA molecules come in the shape of a double helix. In their, now classical paper in Nature, March, 1953, Watson and Crick stated "it has not escaped our notice that the specific base pairing we have postulated immediately suggests a possible copying mechanism for the genetic material".

The helixes can be thought of as twisted ladders or a spiral staircase with the rungs made up of four different chemicals known as nucleotide bases. The chemical names of these four nucleotides are **Adenine, Guanine, Cytosine**, and **Thymidine**, abbreviated **A, G, C, T**. These four letters form the alphabet of the language of life. These chemical bases are always arranged in pairs. When A occurs in one strand T occurs opposite on the other strand, G pairs with C. This is known as the complimentary base pairing. Thus the arrangement of sequences of bases in one strand determines the sequences of bases in the opposite strand. The two strands are said to be complimentary.

A, G, C and T when strung together compose "sentences" or genes or sentences in the language of life. A gene confers, for example blue eyes for one and brown eyes for another. A typical gene has about 1000 base pairs, some may have as many as 200,000. In contrast, the **human genome**, that is the total DNA carrying all the genetic information and non-genetic sequences is about 3×10^9 base pairs. It should therefore be apparent, that looking for a human gene in this huge and complex molecule, is comparable to the proverbial job of searching for a needle in a hay stack!

As predicted by Watson and Crick in their paper in 1953, the complimentarity of two strands is the fundamental mechanism allowing for transfer of genetic information from parent to the offspring. Following the separation of two parent strands a new complimentary strand is synthesized using each of the parent strands as templates, resulting in two daughter duplex DNA molecules (Figure. 1).

Humans are believed to carry approximately 50,000 genes corresponding to 50,000 proteins, all of which are not yet characterized. The definition of rDNA as adopted by the Gene Manipulation Advisory Group (GMAG) of Britain says "the formation of new combinations of heritable material by the insertion of nucleic acid molecule, produced by whatever means, outside the cell, into any virus, bacterial plasmid, or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of propagation".

Why Use Gene Technology

There are several reasons for the need to clone genes:

i. The DNA content of higher organisms is enormously complex. As previously described the DNA content of each human cell is 3×10^9 base pairs.

ii. The isolation of individual genes by conventional methods is impossible because,

   a) of the relative low abundance. Most genes are presented only once in every cell. Human genome contains approximately 50,000 genes.

   b) great deal of DNA has no apparent function

iii. The chemical homogeneity of genes. The only difference between two genes is in the nucleotide base sequence. No known physical or chemical method can effectively distinguish between two genes.

The technology of gene cloning is able not only to overcome the above constraints but also provide means for the amplification of genes and gene products.

Denaturation and Renaturation of DNA

Another important property of DNA arising from complimentarity,

which is applied in almost every gene technology experiment is known as denaturation and renaturation of DNA. When the DNA molecule is heated to 100^{0} C, the two chains separate out. This is known as denaturation. If it is allowed to cool gradually then the two chains once again form the double stranded structure due to complimentarity of two chains. This is known as renaturation (Figure 2).

Restriction Enzymes

An important landmark in the present day development of gene technology is the discovery of restriction enzymes. Certain proteins - called enzymes - that "chew up" the DNA of viruses invading bacteria, thereby restricting the growth of the virus and protecting the bacterial cell, turned out to be nature's chemical "scissors" and essential tool for the genetic engineer. These special proteins attack and cut DNA molecules at specific sites. There are many kinds of restriction enzymes. Some are specific for a sequence of four nucleotides, some for five and others for six. When a cleavage is required at an exact spot along a DNA molecule, the proper enzyme is selected to do the job. By using these chemical scissors, specific sequences of DNA - genes - can be cut out of a complex DNA molecule resulting in a discrete set of fragments.

Vectors

The discovery of double stranded circular DNA molecules in bacteria replicating independent of the bacterial chromosome is another landmark in the development of gene technology. These are known as **Plasmids.** These plasmids were also found to carry genes which confer upon the host bacterial cell the resistance to antibiotics such as tetracycline, empicilline etc. Thus these plasmids while coexisting within the host bacterial cell, also provide means of survival for the host in adverse conditions such as exposure to antibiotics. These plasmids became the genetic engineer's molecular vehicle for the transport of a foreign gene into the host bacterial cell. Nowadays, not only the plasmids but bacterial viruses, known as **phages** are also used in gene cloning experiments.

Gene Cloning

A typical approach of gene technology to isolate a human gene of

interest is illustrated in figure 3. This approach is also known as gene cloning because it leads to the isolation of a clone of bacteria carrying the gene of interest. Following the cloning of a gene, the next step involves the identification of the gene of interest. The basic steps involved are outlined in figure 4.

How can this Technology Contribute Towards Development?

Let us now ask the question how this technology can contribute towards development? For any development strategy to be successful the following requirements may be considered an a priori.

a) Healthy nation
b) Viable agriculture
c) Viable industry

Healthy Nation

In order to provide a healthy population the following facilities are essentiai:

a) Efficient methods of diagnosis of diseases,
b) Effective drugs for the treatment of diseases,
c) Effective methods for the prevention of diseases,

If one considers Sri Lanka, the major diseases affecting the population, thereby adversely affecting the development plans both already implemented and those ear-marked could be classified as:

i. Infective diseases:

a) Parasitic diseases eg. Malaria, Filariasis,
b) Bacterial diseases eg. Diarrhoea, Venereal diseases,
c) Viral diseases eg. Japanese encephalitis, hepatitis,

ii. General pathological disorders of the human body:

a) Respiratory diseases,
b) Heart diseases,
c) Cancer.

iii. Genetic diseases

The effective management of these diseases requires reliable methods of diagnosis, treatment and prevention.

Potential of Gene Technology to Improve Diagnostic Methods

In order to illustrate the application of genetic engineering to improve diagnostic methods, it is convenient to consider malaria and filariasis. The most conventional method of malaria diagnosis is by microscopy, where a sample of blood taken from the patient is examined for malarial parasite under a microscope. Similarly for filariasis, a sample of blood collected between 2100h - 2400h is examined for *microfilariae* microscopically. The advantage of this method is that no sophisticated instruments are required except a microscope and a trained microscopist. However, if the parasitaemia is very low, as in the case of malaria, the technician may have to spend 30-40 minutes per patient for accurate diagnosis or otherwise the diagnosis may be missed. In view of the reported resistance of *Plasmodium falciparum* to some antimalarial drugs, accurate detection of the species of the malarial parasite is essential for instituting effective treatment. Furthermore, when the prevalence of the disease reaches epidemic proportions, the availability of technicians and the time required for microscopic examination may become limiting in rapid diagnosis and treatment.

Recent advances in genetic engineering have made it possible for the handling of large number of (100-200) samples of blood within a day with very high sensitivity. As described previously, the DNA molecule is unique to all living organisms. Within this unique molecule, there are also sufficient differences at the molecular level capable of differentiating between even two types of malarial parasites. Using genetic engineering techniques, such species specific DNA fragments have been cloned, amplified and developed as DNA probes for the diagnosis of malaria and filariasis. These fragments known as **repetitive sequences** occur in the parasite genome in high copy numbers. Using these DNA probes and the technique of nucleic acid hybridization, based

on the property of denaturation and renaturation, it is possible to detect the parasite DNA in human blood thereby diagnosing the disease and also establishing the species of parasite.

The advantages of the DNA based technique are the species specificity of the probe and the ability to handle several hundred samples in one day. The disadvantage is the need to use radio-isotopes. However, nonradioactive detection methods are also currently available, but with less sensitivity than radioactive detection methods. Both the researchers all over the world and the companies developing diagnostic methods are fully aware of the need for nonradioactive labelling procedures in order to make this technology more accessible to the developing countries as well as for field applications. The developments in this field are taking place at rapid speed and a nonradioactive method with increased sensitivity is a certainty in the near future.

DNA probes also will be very useful in the vector control programmes and epideomiological surveys. In particular, the present vector control programmes in filariasis involve the collection of mosquitoes from endemic areas, followed by microscopic dissection to look for L_3 larval stage of *Wuchereria bancrofti,* the filariasis causing parasite in Sri Lanka. This is a very laborious and time consuming process and requires expertise. In contrast, a *W.bancrofti* specific DNA probe, can detect L_3 larval stage by DNA hybridization techniques. Here again a large number of mosquitoes can be screened within one day. Such probes have been already developed for *Brugia malayi* and *Brugia pahangi* the human and animal filarial parasites present in other countries of the region.

Genetic Diseases

The classification of genetic diseases is given in Table 1. The majority of these diseases are serious, none are curable and relatively few are treatable. Thus prevention is the only alternative. This requires detection of carriers of defective genes, genetic counselling, prenatal diagnosis and selective therapeutic abortions.

Until the development in genetic engineering during the last decade (1978-1987), the techniques available for the diagnosis of genetic diseases were

karyotyping and biochemical methods. The technique of karyotyping can detect major chromosomal aberrations only. This technique cannot detect molecular defects at the molecular level of the gene. The biochemical methods were also inconclusive as they depend on gene products. Both these techniques were also not applicable in the detection of the carriers of defective genes, particularly X-linked recessive diseases. Thus prenatal diagnosis of genetic diseases such as Duchenne Muscular Dystrophy, X-linked mental retardation and Thalassaemia, Phenylketone urea could not be undertaken with any hope of success.

With developments in gene technology, in contrast, almost fool-proof diagnosis and carrier detection of some of the most lethal genetic diseases can be carried out today. These include Duchenne Muscular Dystrophy, - progressive degeneration of muscles resulting in death in the third decade of life -, Thalassaemia, Hemophillia and several others. Over the last few years molecular biologists and clinical geneticists have concentrated their efforts in cloning and amplification of DNA fragments from the human genome which are closely linked with a given genetic disease. These DNA fragments serve as tools for diagnosis and carrier detection of defective lethal genes. Using these DNA probes and chorionic villi samples from as early as 7 weeks of foetal growth, it is possible to predict with excellent accuracy whether a foetus is carrying a lethal genetic defect. A decision then could be taken to resort to therapeutic abortion. A list of currently available gene probes is given in Table 2.

Heart Diseases

Cholesterol, atherosclerosis and heart diseases are very popular topics today. What is perhaps not very well known is the fact that two biomedical scientists working in close collaboration for over two decades at the University of Texas Medical school, on cholesterol metabolism became the recepients of the Nobel prize for Medicine and Physiology in 1985. The ultimate scientific achievements of these two scientists, Goldstein and Brown, was the application of gene technology to the evaluation of molecular mechanisms of cholesterol metabolism and how derangements in the molecular mechanism, could lead or predispose one to heart disease.

The contribution to our understanding of the molecular mechanism of cholesterol metabolism in the human, makes it possible to predict the predisposability of one to the development of heart disease or whether one carries

a genetic defect which would lead to an untimely death early in life. That is the state of the art as it exists today, but with the rapid advances taking place, it may well be that gene replacement therapy becomes available soon, perhaps with the advent of the 21st century. This may provide the patient carrying a completely defective gene in cholesterol metabolism or for any other genetic disease, to be replaced with the correct gene.

Drugs and Other Pharmaceutical Products

Genetic engineering now allows biological synthesis and large scale production of several proteins. The first such therapeutic agent to be produced in large scale using a genetically engineered micro-organism was human insulin which came into the market as early as 1979. This was a milestone in the success of gene technology. This achievement also led to massive investments by most pharmaceutical companies on gene technology related projects with a commercial potential. In less than a decade these investments have brought unexpected dividends running into millions of dollars. Table 3 gives some of the pharmaceutical products currently produced genetic engineering methods, and all are used in the treatment of human disease. If not for the technological break throughs of genetic engineering, no entrepreneur would have ever dreamt of investing in the commercial production of most of these pharmaceutical products. Thus the fate of hemophiliacs today is much more promising with the availability of blood clotting factors VIII and IX, and need not depend on regular blood transfusions. The adverse effects of such transfusions are well known, particularly the risk of infection by AIDS virus.

Potential of Gene Technology for the Production of Human and Animal Vaccines

In the field of development of vaccines for both human and animal diseases, gene technology provides an immense potential. Techniques for production of efficient vaccines have been known for more than a century and it is today a well known fact that smallpox has been eradicated from the earth due to a successful vaccination programme. Also the poliomyelitis vaccination has been of tremendous importance to mankind. The classical methods for vaccine production are however in many ways cumbersome and expensive, since the production of animal virus vaccines requires large scale growth of tissue culture cells. Some micro-organisms which are of great medical significance are,

moreover, difficult to propagate under laboratory conditions. It is anticipated that genetic engineering could circumvent these shortcomings and be used to produce efficient vaccines.

Vaccines produced by recombinant DNA technology would be expected to have a number of advantages as compared with vaccines produced by conventional methods:

a) The vaccine will not contain the entire infectious agent, thus eliminating the risk of spreading infections during the manufacturing process or by the handling of large volumes of the infectious agent. This problem is of considerable importance for instance, in connection with production of vaccines against hepatitis B virus and foot and mouth disease virus.

b) No inactivating processes are required and loss of activity due to deleterious effect of the inactivating process is avoided.

c) No test of innocuity of the vaccine is required at least in terms of residual activity.

d) Technically demanding large scale cultivation of mammalian cells is not required.

e) Considerably larger quantities of any vaccine could be produced by recombinant DNA technology. Hence, shortcomings in potency may be overcome by scale of production.

f) Handling of the vaccine will be less demanding since vaccines can most likely be produced which do not require refrigeration and the same careful handling as conventional vaccines. This will facilitate storage and distribution.

g) Production costs are likely to be cheaper and the vaccine supply will essentially be unlimited, once a suitable recombinant strain has been constructed for vaccine production.

The principle behind the use of genetic engineering for vaccine production is that one or a limited number of genes from the pathogen in question are

inserted into a vector which then is transferred to a suitable host for expression. A prerequisite for the construction of vaccine producing strains is that the gene is known that encodes the antigen or the antigens which induce protective antibodies.

Already great strides have been made towards the production of a vaccine against malaria caused by *Plasmodium falciparum* the most lethal form. Several stage specific target antigen genes have been cloned and characterized. Of these, the most promising one has been the gene coding for the circumsporozoite protein of the parasite. Antibodies raised against synthetic peptides or recombinant proteins expressed in bacteria have been shown to inhibit sporozoite invasion of liver cells. Several leading groups all over the world are concentrating on the production of a successful vaccine against malaria and it may well be that such a vaccine becomes available before the end of this century.

Agriculture

Sri Lanka, like most other developing countries in Southeast Asia, has an agricultural based economy. Our staple diet is rice. The major foreign exchange earning commodities are tea, rubber and coconut. The potential of gene technology in increasing the productivity and the quality of these agricultural products is tremendous. The productivity of these agricultural products is at the mercy of natural phenomena such as rainfall, susceptibility to pests and the availability of fertilizers. The extremely high cost to the national exchequer on the importation of synthetic fertilizers such as urea and the pesticides and weedicides needs no emphasis.

The technology of genetic engineering provide, perhaps an unlimited potential, if only properly harnessed in making this nation self-sufficient with its food requirements.

Chemical control of insect pests is estimated to cost more than US$ 3 billion per year worldwide. Over US$ 400 million is spent each year for control of *Lepidopteran* pests in the US alone. The genetic engineering of insect tolerance into crop plants is therefore a goal of significant interest to agricultural biotechnology. *Bacillus thuringiensis* (B.t.) is an entomocidal spore-forming bacterium. Most strains of B.t. are specifically lethal to *Lepidopteran* insects

e.g. larvae of moths and butterflies. This insecticidal activity resides in a protein produced by B.t. The Scientists at Monsanto Company, USA, have successfully introduced this gene for the protein into tomato plants to produce what are called transgenic tomato plants. These transgenic tomato plants expressed the foreign gene of B.t. and plants were resistant to *Lepidopteran* insects.

The damage caused by frost to agriculture is another major problem in temperate countries. It is known that certain bacteria prevalent on plant leaves, especially *Pseudomonas syringae* produce a protein that facilitates the nucleation of ice crystals, leading to frost damage. Stephen Lindlow at the University of California, Berkley, USA, has genetically engineered a strain of *P. syringae* that lacked the gene for the ice nucleating bacteria on leaves and thereby lower the temperature at which plant would suffer from frost damage.

Scientists at the Biotechnica International, USA, have developed nitrogen fixing bacteria which enhance the growth and yield of alfafa. In these developments, nitrogen fixing genes from *Rhizobium meliloti* were used to produce genetically engineered organisms. The technology presently available also makes it possible to improve the quality and quantity of proteins in staple food crops. John Dodds (Lima) has shown how the insertion into potatoes of an artificially constructed gene coding for protein rich in essential amino acids could reduce from 1.8 to 0.8 kg, the amount of potato that one must eat each day to get the proteins one needed. These developments raise the hopes of millions of unfortunate people in developing countries where protein calorie malnutrition is rampant. In majority of the third world countries the staple diet is rice in which the essential amino acid, lysine and to lesser extent methionine, is deficient. The development of new varieties of rice to which genes coding for proteins of high nutritive value have been genetically engineered is within the present capability of gene technology. The production of drought resistant, high yielding varieties of major economic crops of Sri Lanka such as tea, rubber and coconut, and other minor export crops are areas where high priority should be given.

It was a realization of the immense potential of genetic engineering and biotechnology on the development of less developed countries, that the United Nations Industrial Development Organization (UNIDO), established the International Centre for Genetic Engineering and Biotechnology (ICGEB), partly in New Delhi, India and in Trieste, Italy.

In this review, I have attempted to outline the basic concepts of gene technology in a language apprehensible to a majority of people, including administrators, planners and especially politicians. Following this, I have highlighted the areas where developments have been extremely fast but with strong impact on developing countries of the world.

However, it should be emphasized, that the road to achievements of such goals are easily said than done. If Sri Lanka is to benefit from this rapidly advancing technology, a **national commitment** at the highest level is a *sine qua non.* It is also relevant to focus at this juncture, on the enormous investments in this area currently made by the multi-national companies in developed countries. The discoveries are patented faster than they are discovered. This is not unusual when one realizes the quantum of investment made on this technology. It is therefore absolutely essential that a national commitment is made with no further delay in the formulation of a national programme on gene technology and biotechnology with adequate funds to undertake research and development in priority areas.

The expertise available locally at the present time, although minimal, is capable of undertaking the initial projects. The Department of Biochemistry, University of Colombo, took a major leap in this respect in 1984, to establish a unit on Molecular Biology and Gene Technology with collaborative assistance from the University of Uppsala, Sweden, a leading scientific institution in this field. Under this programme several research projects relevant to the development strategy of Sri Lanka were identified and presently under investigation. A postgraduate course in Biochemistry, Molecular biology and gene technology at the level of MSc was initiated in 1986. This is aimed at the training of manpower resources to undertake research in gene technology relevant to Sri Lanka. However, the resources available for the expansion of the activities of this unit are now becoming limited. The first batch of MSc graduates with sufficient training in gene technology were expected to pass out in November 1988. This group forms the nucleus of the genetic engineers in Sri Lanka. It will be very unfortunate if these young trainees could not be retained in the country, to undertake further advanced research on problems relevant to Sri Lanka, leading towards a PhD degree. The laboratories are fully equipped with all essential advanced instruments but limited by the space available and the funds to meet the recurrent budget and salaries of these young scientists dedicated to contribute with their training towards the development of

their motherland.

Acknowledgements

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Table 1

Classification of Genetic Diseases

Type	Approximate Number
Autosomal Dominant	736
Autosomal Recessive	521
X-linked Disorders	107
Additional suspected unifactorial inheritance	1447
Total	2811

Table 2

Some Genetic Diseases for which Gene Probes are Available

Disease	Gene Probe	Year of Discovery
Sickel cell anaemia	B-Globin	1978
Dwrafism	Human Growth Hormone	1982
Lesh-Nyhan Syndrome	HPRT	1982
Heart Disease	LDL receptor	1983
Alpha Thalassaemia	Alpha Globin	1983
Beta Thalassaemia	Beta Globin	1983
Hemophillia A	Factor VIII	1986
Hemophillia B	Factor IX	1986
Familial Hyper-Cholesterolaemia	LDL recepter	1986
Duchenne Muscular Dystrophy	X-chromosome fragments	1983
Cystic fibrosis	Chromosome 4&19 fragments	1986
Phenyl keton urea	Phenylalanine hydroxylase	1984
Hyperlipidaemia	Aplipoprotein C	1986
Abetalipoproteinaemia	Aplipoprotein B	1986

Table 3

Some Pharmaceutical Products from Gene Technology

Product	Year of Production
Somatostatin	1977
Insulin	1982
Growth hormone	1979
Hepatitis B vaccine	1982
Interferon-alpha	1983
Inteferon-alpha-2B	1984
Tissue plasminogen activator	1983
Factor VIII	1986
Apolipoprotein B	1986

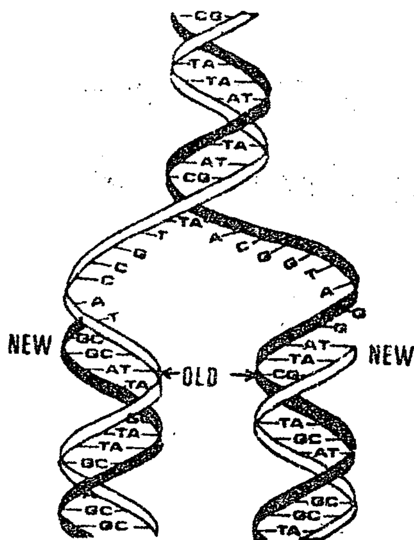


Figure 1 Double helical Structure of Deoxyribo Nucleic Acid

Note Complimentary base pairing, A with T and G with C. When a daughter molecule is to be produced, the parent strands (old) separate out and acts as a template on which new strands are synthesised according to complimentary base pairing.

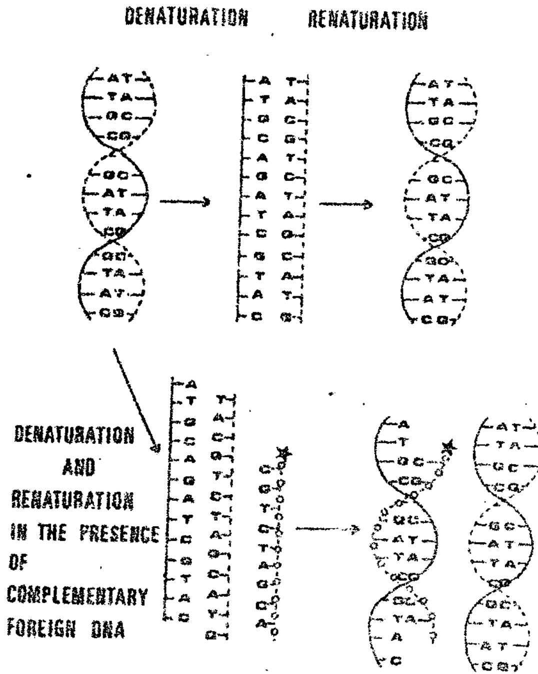


Figure 2 Denaturation and Renaturation of DNA

When DNA is heated to 100°C, the two strands separate (denaturation), on cooling the two strands join together (renaturation). As shown in the lower part, a foreign peace of DNA with complimentarily can compete with the two original strands for renaturation.

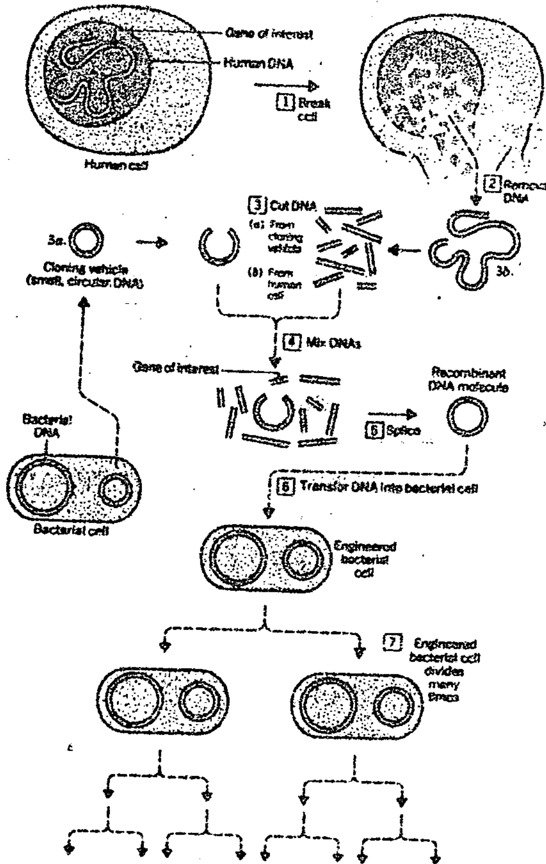


Figure 3. Major Steps Involved in Gene Cloning

(1) Human cells are broken. (2) DNA containing the gene of interest is removed from human cells. (3) The DNA from cloning vehicle and human DNA are cut in specific places. The cloning vehicle DNA is obtained from bacterial cells. (4) The two types of DNA are mixed. (5) The DNA fragments are spliced together, yielding a recombinant molecule. (6) The recombinant DNA molecule is transferred into a bacterial cell. (7) The engineered cell created by step 6 is allowed to reproduce millions of times to form a clone of identical cells.

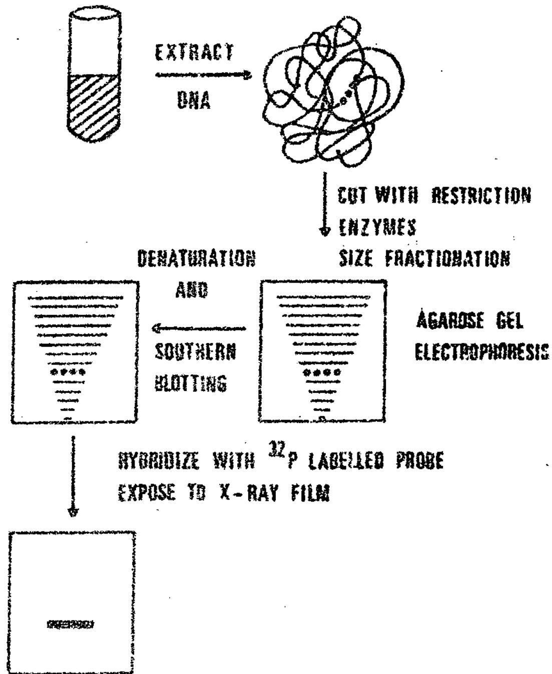


Figure 4 Detection of a gene of Interest

A gene of interest in the human body can be detected, if a complimentary probe is available. The probe is labelled with a radioactive substance, hybridized to gene of interest and exposed to an X-ray film. The autoradiogram will reveal the gene of of interest.

ADDENDUM - 1991

IMPACT OF MOLECULAR BIOLOGY AND BIOCHEMISTRY - A BRIEF NOTE

Prof. Eric H. Karunanayake

The rapid advances made in the fields of Molecular Biology, Biochemistry and Gene Technology have now made it possible to understand the pathology of disease processes at the molecular level.

The greatest impact of these advance have been and will continue to be on prevention, diagnosis and curative aspects of medicine.

To give an example, nothing was known about Duchenne Muscular Dystrophy even upto 1982. Today we know the gene, the entire letter by letter sequence of the gene and the protein that the gene codes. The most remarkable achievement is that one can undertake prenatal diagnosis of this fatal disease as early as 6 - 7 weeks of gestation using chorionic villi samples. That is even before one is fully aware that a conception has occurred. Same can be said of haemophillia A and B, thalassaemias, sickle cell anaemia, cystic fibrosis, Huntingtons chorea, fragile-X syndrome.

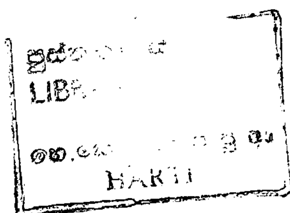
The predisposition to hypercholesterolaemia and atherosclerosis in later life can be predicted with very good accuracy with DNA based techniques. The gene replacement therapy is a definite possibility in the 21st century, if not earlier.

In the case of tropical diseases such as diarrhoea, malaria, filariasis, schistosomiasis and onchocerciasis DNA based diagnostic techniques enables rapid diagnosis, evaluation of resistance to drugs and epidemiological surveys. Development of new drugs based on the molecular characteristics of parasites and bacteria, and specific chemotherapeutic targets as well as synthetic vaccines developed by genetic engineering techniques are on the way.

Hormones such as insulin, growth hormone and somatostatin as well as clotting factors developed by genetic engineering are already in the market and many more will reach the pharmacy soon.

Sequencing and mapping of the entire human genome is already underway. At the NIH, a special office called "office of the Human Genome Mapping" is already established with Nobel laureate James Watson as the Director, and a billion dollar budget. In the EEC, Human Genome Organization (HUGO) is already functioning. The mapping and sequencing of the human genome will have an unforeseen impact on Biomedical Sciences. The impact of the biological revolution on the human society, disease process, care and its prevention will far exceed anything experienced after the industrial revolution and electronic-computer revolutions.

Should we then give highest priority to molecular biology and biochemistry in teaching basic sciences in the medical curriculum to equip the new generation of doctors with adequate knowledge to cope up with challenges awaiting them?



RESEARCH TOWARDS MALARIA VACCINES

Shiroma Handunnetti, MBBS, Ph.D and
Professor Kamini N. Mendis, MBBS, Ph.D
Malaria Research Unit, Department of Parasitology
Faculty of Medicine, University of Colombo

Research on Malaria vaccines is at a stage where sub-unit constructs are being developed and evaluated for their potential to elicit an immune response in man. One approach, that of anti-parasite vaccines, aims to induce immunity against the parasite and the other, anti-disease vaccines, against parasite components which evoke immunopathology in man. A growing list of parasite components of sporozoites, asexual blood stages and sexual stages in the mosquito are being characterized as potential anti-parasite vaccine candidates. Genes coding for most of these antigens have now been cloned. Many have been evaluated in vaccine trials in non-human primates and even man. Sporozoite vaccine trials in man with what was thought to be a promising vaccine candidate resulted in a disappointing immune response in vaccines. This has raised fundamental questions as to whether the immunological effector mechanism in man against sporozoites is mediated by humoral antibodies or whether it is mainly a cellular response, and also some fundamental questions about immunogenicity of malarial antigens. In the field of asexual vaccine development, on the one hand is a partially successful vaccine trial in humans. The vaccine comprises of a synthetic polymer of regions of three sexual blood stage proteins, two of which whose location in the parasite is still not known. On the other hand are a large number of antigens structurally well defined and characterized, and in many of which the genes have been cloned but with their candidature being still questionable. With respect to the vaccines against sexual stages the transmission blocking vaccines present a straightforward antibody mediated mechanism. Several candidate antigens have been recognized and some cloned, yet others because of the confinnational nature of their epitopes may require eukaryotic expression. These various vaccines are based on the classical approach to vaccination which is to raise host immunity against the parasite so

as to reduce parasite densities or to sterilize an infection.

The availability of cloned and expressed parasite antigens has led to a significant body of knowledge on the development of immunity to sub-units, the field today presents a series of problematic issues. The critical ones among them are, the selection of vaccine candidates from among a range of parasite molecules, restriction of the host immune response due to MHC and other forms of host polymorphism and immune evasion mechansims of the parasite such as antigenic polymorphism. Recent studies have also focused on immuno-pathological pathways in malaria and the identification of candidate antigens for anti-disease vaccines. Other critical issues facing malaria vaccines are related to expression and delivery of antigens. Eukaryotic expression may be essential for certain malaria antigens. A range of live vectors, virus and bacterial, are being explored as delivery systems. Thus research carried out during the past decades has laid an extensive foundation of relevant knowledge for the development of a malaria vaccine. A continued effort will be required to attain this objective as the goal itself remains as important as ever. Will the funding agencies as well as scientists remain committed to this objective?

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GENETIC IMPROVEMENT THROUGH INDUCED MUTATIONS

P. Ganashan, B.Sc(Agric.), Ph.D.
Director, Plant Genetic Resources Centre,
Department of Agriculture, Peradeniya

Introduction

The manipulation of the biological system - biotechnology - has been described as the latest technological revolution of the 20th century. Although the basic techniques of biotechnology have been developed and known in the past, the effective application of these techniques in genetic improvement work is a relatively new avenue for research. Among the various biotechnological approaches for the improvement of crop plants, changing the genetic factors leading to the alteration of expression of their characteristics form the major approach in recent times.

From ancient times, farmers have contributed to the modern day agriculture through the simple method of selecting superior plant types, which often arose as spontaneous mutations. To hasten selection, scientists have developed plant breeding methods to evolve desirable varieties combining high yields, resistance to biotic and abiotic stresses etc. from the available germplasm. However these methods are considered slow and cumbersome as many years are spent to crossbreed and develop different varieties. As these conventional technologies alone may be inadequate to meet present and future challenges, we must integrate recent advances in biotechnology with the techniques already in use.

The science of genetics was born with the experiments of Gregor Mendal, who proved in 1866 the intact transmission of hereditary factor called genes from one plant generation to another. In 1895, nearly 30 years later X-rays

were discovered by Roentgen. However it took another 30 years to prove that radiation causes heritable changes in plants. In 1927 H.J. Muller discovered that mutation rate is greatly increased by X irradiation, for which Muller received a Nobel prize. This has resulted in the development of mutation induction technology.In the 1940s Oswald Avery, and his colleagues discovered that genes were in fact made of deoxyribonucleic acid (DNA). In 1953 James Watson and Francis Crick found that DNA was a double helix, two intertwined strands each composed of chains of four different chemical bases called : (1) Adenine, (2) Guanine which are purines, (3) Thymine and (4) Cytosine which are pyrimidines.

Considerable advances were made in understanding the genetical effects of radiation. Physical mutagens like X-rays, gamma-rays and neutrons (fast and thermal) were soon complemented by chemical mutagens as Ethyl Methane Sulphonate (EMS), Diethyl Sulphate (DES), Ethyleneimine (EI), Ethyl Nitroso Urea (ENU), Methyl Nitroso Urea (MNU) etc. Joint FAO/IAEA Division formed in 1964 assisted in accelerating the induction of mutations for crop improvement. Before 1950, there was only one variety derived from induced mutation. In 1969 there were around 100 varieties. Now another 20 years later, world wide at least 1300 cultivars were derived from induced mutations. This indicates that the technology of mutation induction has been accepted as a valuable additional tool to create improved cultivars. Induced mutation programmes were carried out in Sri Lanka from 1966 to evolve high yielding cultivars of food crops and pasture grasses.Rapid advances in the science of Genetics, and the development of *in vitro* technologies have provided a new dimension to the techniques of mutagenesis.

Of the new technologies (a) clonal propagation, (b) *in vitro* conservation, (c) pathogen-free plant production, and (d) molecular diagnostics (to produce or screen clonal material) are relevant to conventional crop improvement programmes and are mostly used with the available genotypes. For the development of *in vitro* - derived germplasm, the relevant technologies are, (a) embryo rescue, (b) somaclonal variation, (c) anther culture, and (d)non-sexual gene transfer (protoplast fusion, use of bacterial or viral vectors, and direct gene transfer through microinjection or electroporation, etc.). *In vitro* techniques, and the production of haploid plants through anther culture are employed to provide more effective and rapid results in mutagenesis.

Green Revolution in *Indica* Rice Through a Spontaneous Mutant

Traditionally plant breeders used the existing genetic variations within and among species as sources of variations in their hybridization and recombination work. Thus genetic improvement work was limited to the existing variations. For instance, improvement of *indica* rice varieties could not make head way before the 50s, and any improvement by *indica-japonica* hybridization programmes did not give the desired results. The genetic variability that existed within the *indica* sub species was unable to boost the yield levels by responding to added fertilizers. On the other hand the variability that existed in the *japonicas* cannot be easily made to combine with the *indicas.*

The discovery of the spontaneous dwarf mutant Dee-geo-woo-gen in the *indica* rice in China changed the picture. This mutant became the starting point of the *indica* yield revolution and was extensively used in hybridization programmes in the South and South East Asian countries, which resulted in semi dwarf *indica* varieties like Taichung Native-I of Taiwan and IR-8 of IRRI, responding to high fertilizer doses completely dispelling the impression that only *japonica* strains could respond to higher fertilizer inputs.

Instances of spontaneous dwarf mutants in rice were also observed in Sri Lanka. The spontaneous dwarf mutant, namely K8-mutant was isolated from H4 during 1966, in the Hambantota District (Somapala 1967). Its dwarfing gene is different from that of the Chinese *indica* dwarf Dee-geo-woo-gen.

Significance of Induced Mutations

Mutations arise as a change in the sequences of the four bases which provide the informational content of DNA. Mutations can also result from the addition or deletion of one or more base pairs. Mutations are the main source of genetic variation within a population, and this genetic variation is the raw material of evolution. The significance of induced mutations depends largely on the question of whether a higher total mutation rate can be obtained, and whether the output of useful mutants can be increased. The final output of mutants depends on the technique of original induction, the size of the population handled, and the selection pressure imposed.

Although induced mutations are by and large random, and many of them are deleterious in the environment in which they appear, the pertinent benefits derived are mainly:

a) It is the only method by which characters can be created that are non existent in the natural population.

b) It brings about desirable changes in crop plants without significantly affecting other useful traits inherent in them in a shorter period than by the conventional breeding method involving hybridization and selection.

c) It is the only method by which tight linkages can be broken with beneficial effects.

Plant Materials for Treatment

Plant materials can be treated in any form. Whole plants can be treated in gamma-fields, or gamma-rooms, while small plants, seedlings, tubers, bulbs, corms, vegetative cuttings etc. can be treated with gamma source, or with other sources of radiation. Meiotic cells are more radiosensitive than mitotic cells, and hence it is advisable to irradiate plants in the flowering stage in order to affect the developing gametes. Seeds are commonly used for irradiation, and also for treatment with chemical mutagens, where the apical meristem or embryo will be affected.

Tissues and cell cultures can also be treated with mutagens. There are more potential applications of the technique of tissue and cell culture, than originally conceived. Of the various tissue culture techniques, the induction and selection of useful mutants is probably the most promising for our food crop improvement programmes.

Use of *In vitro* Technologies in Mutagenesis

New *in vitro* technologies have provided an opportunity for wider implementation of mutation techniques under certain circumstances. These new technologies are (1) *in vitro* mutagenesis, (2) *in vitro* selection and (3) the use of haploidy.

a) *In vitro* Mutagenesis

In vitro mutagenesis is the induction of mutations of DNA outside of a cell or organism. The gene is cloned and then mutated either by restructuring segments of DNA, localized random mutagenesis or oligonucleotide-directed mutagenesis. In this method relatively small portions of the genome are exposed to the mutagen. Botstein and Shortle (1985) have given a comprehensive review of *in vitro* mutagenesis.

b) *In vitro* Selection

Callus suspension, or protoplast cultures could be used for *in vitro* selection. The culturing techniques are often mutagenic, and by themselves provide sufficient variation. Variability among plants regenerated from tissue culture - somaclonal variation - is found in essentially all plant species. Instances of somaclonal variations in many important crop plants have been reported by Scowcroft and Larkin in 1984 (Table 1). Variations observed here have been recognized as sources of new variations. Scowcroft and Larkin (1988) pointed out that among the array of somaclonal variants derived from tissue culture, the mutants occur as a result of changes at pre-existing loci, but the evidence for the occurrence of entirely new mutants is, only circumstantial. They also pointed out that genetic events, which naturally occur at a very low rate, occur more frequently during tissue culture, and for this reason is given the impression that somaclonal variants creates new genes. These heritable variations could be used to upgrade the genetic base of coadapted agronomically useful cultivars without risk to their basic genetic integrity.

Mutagens also can be added to the culture medium to further enhance the genetic variation available for selection. If protoplasts in tissue culture vary, desirable genotypes could be selected by varying the culture conditions which allow for the survival of only that fraction of the population of mutants adapted to these specific conditions. The following mutant types could be recovered by these methods:

(a) Autotrophic mutants which are capable of growing in deficient media, and synthesizing some substances.

TABLE 1

SOMOCLONAL VARIATION IN ECONOMICALLY IMPORTANT PLANT SPECIES

(From Scowcroft and Larkin, 1984)

Species	Explant	Variant Character[1]	Trans mission[2]
a. MONOCOTS			
Avena sativa	immature embryo, apical meristem	plant ht, heading date leaf striping, awns	S
Triticum aestivum	immature embryo	plant ht, spike shape, awns, maturity, tillering, leaf wax, gliadins, α-amylase	S
Oryza sativa	seed embryo	tiller no., panicle size, seed fertility, flowering date, plant ht	S
Saccharum officinarum	various	eyespot, Fiji virus, downy mildew, culmicolous spot diseases; auricle length, esterase isozymes, sugar yield	A
Zea mays	immature embryo	endosperm and seedling mutants, D. maydis race T toxin resistance, mtDNA sequence rearrangement	S

1 Characters of possible economic importance.

2 S-sexual transmission; A-transmission through vegetative propagules.

b. DICOTS

Solanum tuberosum	protoplast, leaf callus	tuber shape, yield, maturity date, plant habit; stem, leaf and flower morphology; early and late blight resistance	A
Nicotiana tabacum callus	anthers, protoplasts, leaf	plant ht, leaf size, yield grade index, alkaloids, reducing sugars; specific leaf chlorophyll loci	S
Medicago sativa	immature ovaries	multifoliate leaves, petiole length, plant habit, plant ht, dry matter yield	A
Brassica spp. meristems	anthers, embryos,	flowering time, growth habit, waxiness glucosinolates, Phoma lingam tolerance	S

(b) Auxotrophic mutants which could normally grow only with nutritional supplements; and

(c) Resistant mutants which resist specific drugs, antimetabolites, or abnormal abiotic or nutritional conditions.

The mutant tissue or cell lines thus produced should regenerate plants. Selection of salt resistant cell lines would produce plants adaptable in saline soils which are common in some parts of Sri Lanka.

C) Use of Haploidy

Haploidy and doubled haploidy would also assist in, or improve mutagenesis. In the mutagenized haploid cell line or plant, both dominant and recessive gene mutations will be expressed, as haploid has only the gametic number of chromosomes. Doubling the chromosome number, will result in homozygous doubled haploid plants. Tobacco plants with high photosynthetic efficiency were selected before chromosome doubling (Medrano and Primo-Millo 1985). In addition to the mutation of interest, any other mutations inadvertently produced should be rejected. In mutagenized diploid line, selfing to obtain homozygosity is essential which takes more time.

Doubled haploid techniques offer new ways to improve cultivar yields. The time needed to get a new variety using conventional breeding methods in ten generations could be reduced to as few as three generations.

Combining haploidy for expressing mutations, and *in vitro* selection for screening, will provide powerful methods for mutation breeding. Schematic representation of the culture of excised anthers and the development of haploid plants directly by embryo formation, or through haploid callus, and the production of doubled haploid plants is given in Figure 1. However for most of the cereals, good cell culture technologies are not available, and the spontaneous doubling of haploid cultures retard progress in this direction. Nevertheless, significant progress has been made with biochemical selection.

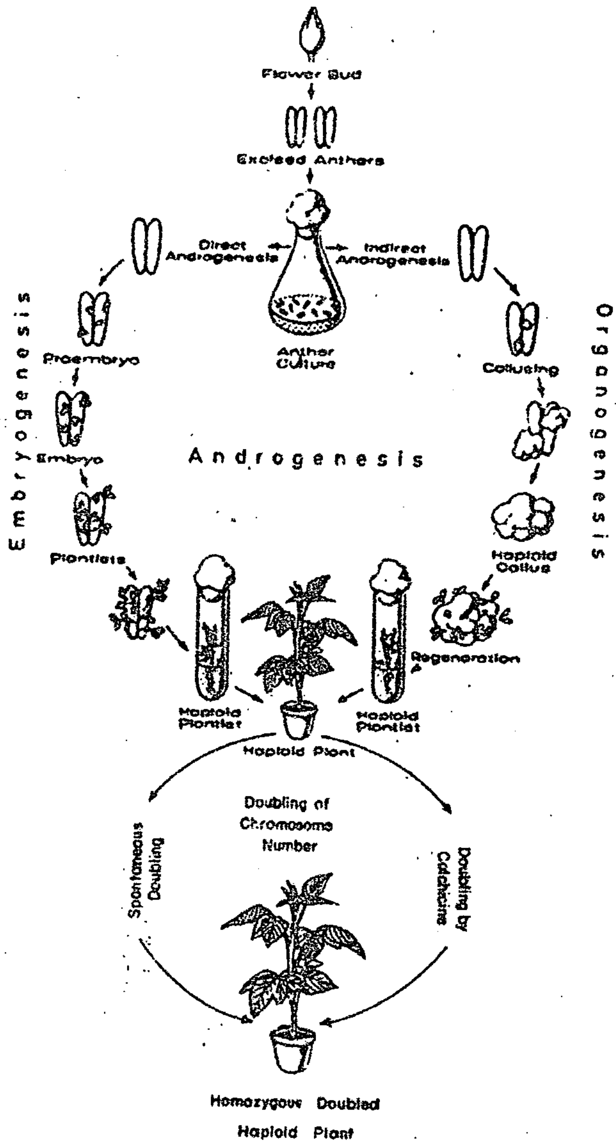


FIG. 01 SCHEMATIC REPRESENTATION OF THE CULTURE OF EXCISED ANTHERS, AND THE DEVOLOPMENT OF HAPLOID AND DOUBLED HAPLOID PLANTS.

Induction of Useful Mutants in Sri Lanka

Sakamoto pioneered mutation studies in Sri Lanka in 1966 using 32P and 60Co gamma rays, but he did not obtain variations from the original type (Sakamoto 1966). Subsequently in Sri Lanka under the project on "peaceful use of atom" of the International Atomic Energy Agency, induced mutation studies were undertaken in rice, sorghum, finger millet, chilli, and pasture grass *Brachiaria brizantha* since Maha 1966/67 season, with a view to obtain desirable high yielding mutants from the popular varieties. Extensive and meticulous mutation breeding studies were undertaken in rice and pasture grasses which had resulted in a wide array of mutants with valuable traits (Ganashan 1969, 1970, 1971). The most promising mutants in rice and *Brachiaria brizantha* have indeed proved the usefulness of induced mutation programmes.

Induced Mutation Studies in Rice

Radiation-induced mutations in rice was first reported by Ichijima (1934) using X-rays and ultraviolet rays, but its practical use in rice improvement was demonstrated by several workers only after 1953 as reported by Gustafsson and Gadd(1966). Futsuhara *et al* (1967) reported the first induced high yielding rice mutant "Reimei" which was released in Japan in 1966 as a national registered variety.

The traditional and popular rice varieties of Sri Lanka have characteristically a tall growth habit, and are susceptible to lodging at high fertilizer levels. In spite of these defects farmers retain these varieties for their qualities. Hence mutational rectification of these was tried at the Regional Agricultural Research Centre (RARC), Maha Illuppallama to obtain desired changes which also has the added advantage of obtaining other desirable traits which are non existent in these varieties, so that the resultant mutants could be used directly for cultivation, or indirectly in recombination and transgression work.

Rice Varieties and Mutagens Used

Four *indica* rice varieties H4, H8, H7 and Pachchaiperumal 2462/11 (PP2462/11) were subject to gamma rays, neutrons and EMS treatments.

Mutagens	Doses
Gamma rays (kR)	0, 10, 20, 35, 50 and 60
Neutrons (R)	0, 300, 600, 900, 1200 and 1600
EMS (%)	0, 0.2, 0.4, 0.6 and 0.8 for 16 hours

Dry seeds were used in all treatments. Dehusked seeds were used for EMS treatments.

The gamma rays and neutron treatments were effected at Stockholm, Sweden and the seeds were brought to RARC, Maha Illuppallama. The EMS treatment was carried out at the RARC, Maha Illuppallama, and the entire mutational experiments were carried out at RARC, Maha Illuppallama.

RESULTS

M_1 Generation

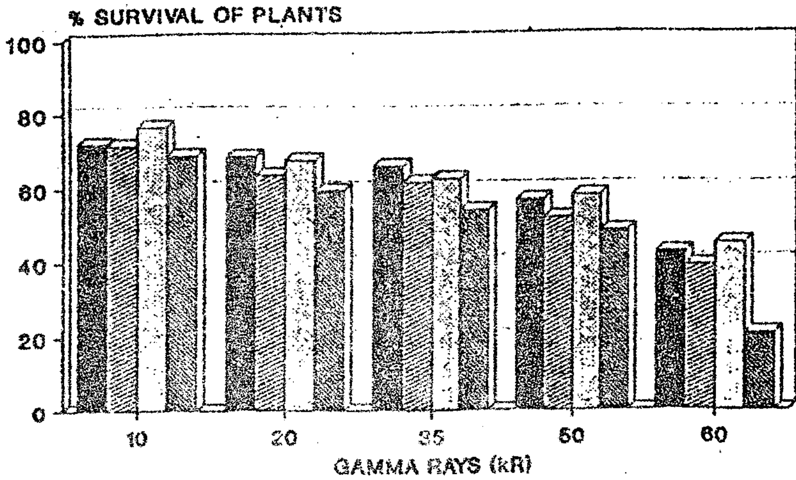
Treated varieties showed different degrees of germination, seeding growth and percentage survival (Figure 2). The variety PP 2462/11 was relatively sensitive to gamma radiation than the other varieties. Chlorophyll chimaeras, stunted and deformed plants were observed in this variety. In the other varieties the trend in sensitivity to survival was the same as for physical and chemical mutagens.In the radiation treatment the gamma ray treatment showed pronounced reduction in the survival of plants compared with neutron treatments.

M_2 Generation

Ten thousand and fifty panicle progenies were grown for the M_2 generation which was screened for chlorophyll and viable morphological mutants. Figure 3 gives the percentage of M_2 lines segregating for chlorophyll mutants in the different varieties of rice. The main types of chlorophyll mutation observed were *albina*, *chlorina* and *xantha* with the *albina* appearing in larger proportion. *Virescens* and *zebrina* were also found in small numbers. The appearance of chlorophyll mutants was independent of the type of

Figure 2

SURVIVAL OF PLANTS



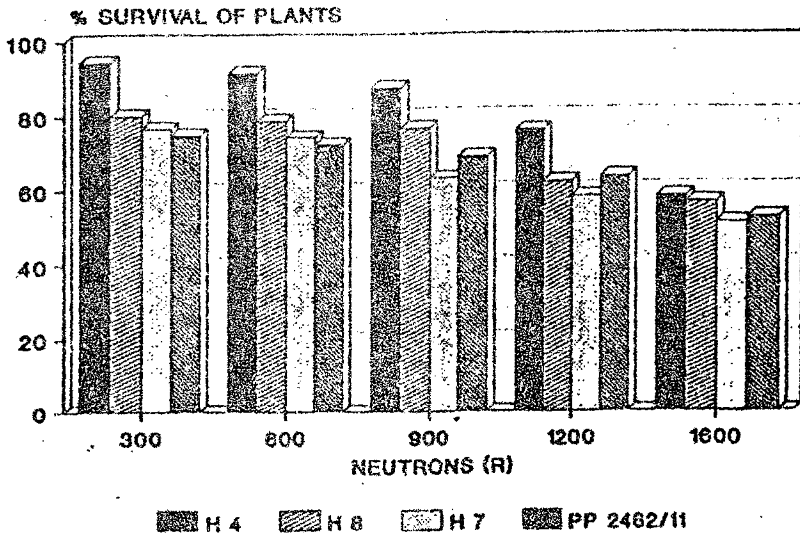
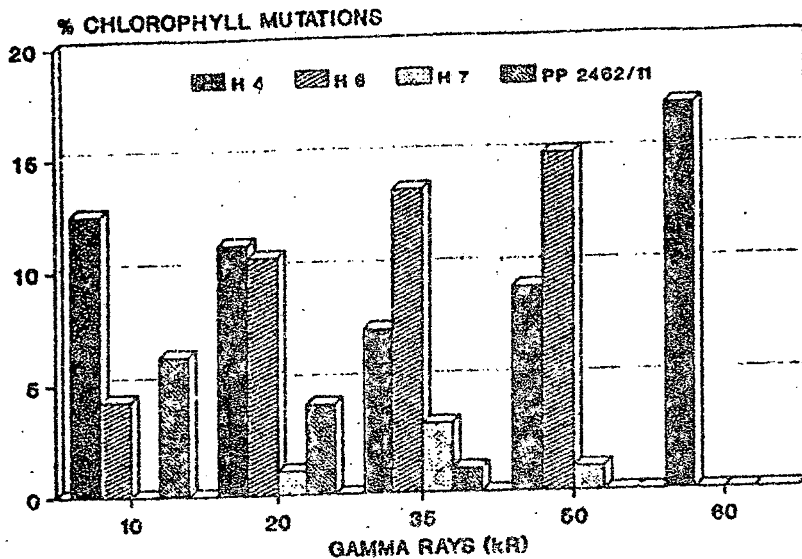
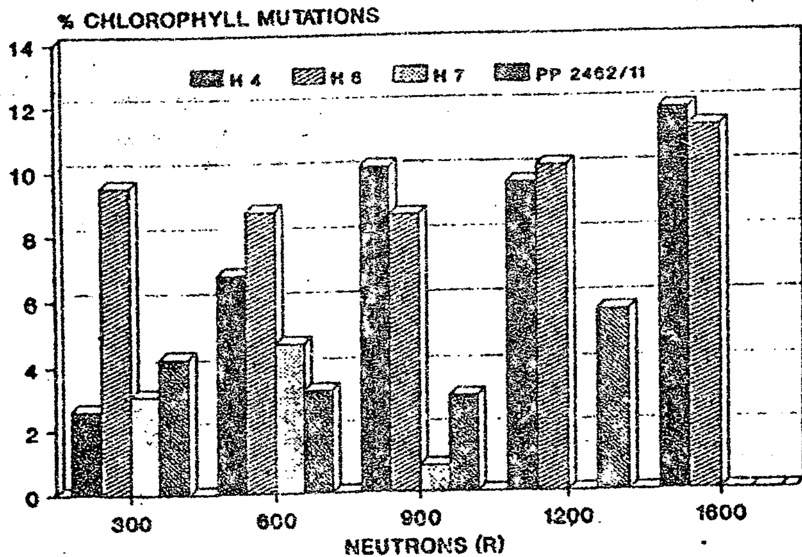


Figure 3

SEGREGATION OF CHLOROPHYLL MUTATIONS





mutagen used. The varieties H4 and H8 showed a greater proportion of chlorophyll mutants than the other two varieties.

A wide range of morphological changes were noticed in the M_2 with several mutated characters appearing concurrently. Modifications occurred in height, habit of plants, flowering date, panicle and spikelet characteristics. Variations in visible characters of the four varieties subjected to gamma rays and neutron treatments are shown in Table 2. As the changes observed were very complex only some of the more prominent characters were considered in this study.

Only changes in seed size and colour were observed in the EMS treatment, whereas in the other two physical mutagens a wide spectrum of mutations, and several mutated characters often appearing concurrently were observed.

The compact panicle mutant with increased tillering and larger grain number in H8, mutants with reduced culm length, increased tillering and resistance to lodging in H8 and H4, and the reduced grain size mutant of PP 2462/11 are some of the useful mutants isolated during the study.

H8 Mutant Lines

Promising H8 dwarf mutant lines with high spikelet fertility were selected in the M_3 and they were tested for yield in the M_4 and subsequent generations. No significant differences in yield were observed between the original variety H8 and its mutant lines. Selections for quantitative characters were made in the successive generations. In some of the short culm and dwarf mutants the total number of grains per panicle showed an increase. However, grain size was smaller than that of the parent, and was characterised by a reduction in the length, breadth and width of the grain. The reduction in length of the grain in these mutants were more striking.

M_4 Mutant Lines

From the M_3 population of H4 lines, short culm and dwarf mutants with more than 50% spikelet fertility and resistant to diseases were selected, and were grown in larger plots for further observations.

Table 2. INDUCED MUTATIONS IN RICE

Number of M2 plant progenies showing morphological changes

Change of character	H4		H8		H7		PP 2462/11	
	Gamma rays	Neutrons	Gamma rays	Neutrons	Gamma rays	Neutrons	Gamma rays	Neutrons
(a) Culm								
Distinctly shorter	7	6	18	11	2	-	4	1
Distinctly taller	3	4	12	4	-	-	-	-
(b) Nature and duration								
Grass clump types	-	7	1	18	-	-	-	3
Early flowering	4	12	6	17	-	-	-	-
Late flowering	10	28	16	16	12	-	11	4
(c) Panicles								
Lax	18	16	8	14	6	12	8	-
Compact and erect	-	-	13	4	-	-	3	2
Non exerted	7	12	4	3	-	7	23	14
(d) Spikelets								
Awned	36	28	12	10	4	-	-	-
Abnormal grain	4	3	4	2	11	15	12	8
Grain size smaller than control	18	17	28	26	7	9	22	14
Grain size larger than control	-	-	17	14	-	-	3	-
Change in husk colour	-	-	16	8	-	-	14	16

In the M_4, selection were made for high spikelet fertility with relatively compact tillering, erect and darker green leaves, thicker grain size and resistance to diseases. Relative values for important agronomic characters of ten dwarf and short culm mutants compared with the parent variety H4 are given in Figure 4.

In the M_5 some of the promising H4 mutants were tested for yield, and simultaneously selections were made for superior genotypes in the purity maintenance plots. Of the H4 mutants which showed superiority over H4, the mutant selection MI-273(m) originated from 35 kR gamma irradiation treatment recorded the highest yield. MI-273(m) has the following desirable characteristics compared to its parent variety H4:

1. Shorter and stronger culm, and hence a greater resistance to lodging.

2. Relatively erect growth habit with darker green leaves which would permit better use of solar radiation.

3. Larger number of panicles without much alteration in the number of grains per panicle and the size and weight of the grains.

4. Non-lodging and non-shattering habits which lend themselves to mechanical harvesting.

5. Wider ranges of adaptability to different agroclimatic zones.

Genetic Investigations of Dwarf Mutants

H4 and four of its dwarf mutants, MI-273(m) and MI-70(m) with gamma irradiation, MI-52(m) with neutron irradiation, and K8(m) selection, a spontaneous mutant, and their diallel crosses were studied in F1 and F2 generations for the pattern of inheritance of dwarfism and other characters (Ganashan and Whittington 1975).

In spite of the close relationship between the varieties, analysis showed statistically significant differences amongst the genotypes for plant height, culm length, panicle length, tiller number, panicle number, number of primary branches, number of spikelets per panicle and area of flag leaf.

Figure 4

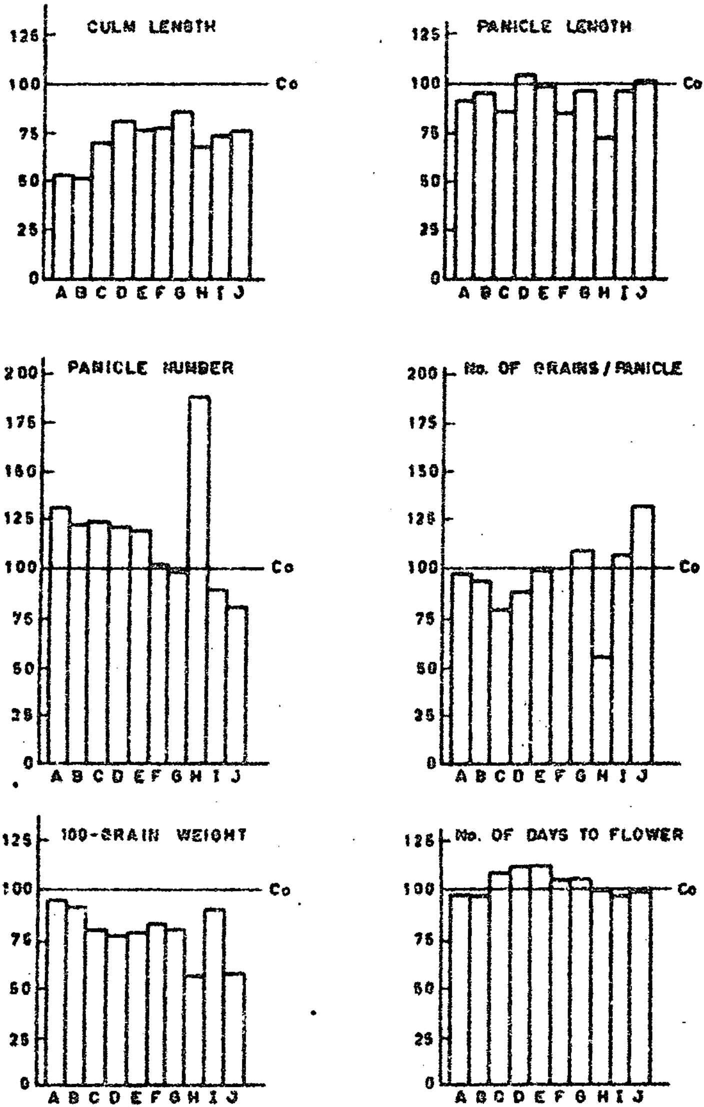


FIG. 4. Relative values for different characters of 10 dwarf and short-culm mutants compared with the mother variety H_4.

Legend. A. M2-273(m); B. 270(m); C. 52(m); D. 644(m); E. 628(m); F. 655(m); G. 656(m); H. 272/4(m); I. 282(m); J. 278(m); Co. H_4.

The High Yielding Mutant Line MI-273(m)

A large number of mutant populations were screened and studied for recovering promising mutants. Of the various mutants obtained, the dwarf mutants of H4 and H8 which appeared promising were studied in detail. The mutant selection MI-273(M) originated from 35 kR gamma irradiation treatment of H4 recorded the highest yields. In the short-culm and dwarf mutants obtained in this study, there is a reduction in panicle length, number of grains per panicle, grain size, and 100 grain weight, with a concomitant increase in panicle number. Similar pleiotropic effect of dwarfing genes were reported by Li *et al* (1966). However the pleiotropic effect of the dwarfing gene does not manifest itself in MI-273(m). Though an almost 50% reduction in culm length was brought about in MI-273(m), it is of interest to note that the panicle number increased by 32%, while other characters, such as panicle length, number of grains per panicle, 100 grain weight and grain dimensions (length and width) remained unchanged (Ganashan 1971).

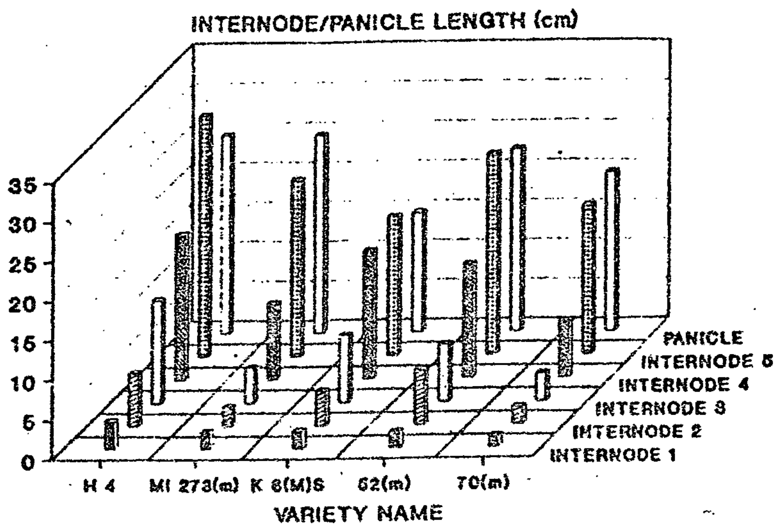
It may be assumed that the variation between the dwarf and the H4 control is likely either to be due to pleiotropic effects of the dwarfing gene or perhaps to the induction of several mutations by radiation procedures.

None of the mutant lines were dwarf because they had a reduced number of internodes. The internodes were shorter than in H4 but the degree of reduction at each internode varied from type to type (Figures 5 and 6).

In a mutation breeding programme involving taller *indica* types to evolve a successful dwarf line, the dwarf mutant should retain or improve relative to the parents its expression of time-to-flowering, spikelet number per panicle, grain weight, and panicle number as well as exhibit the expected benefit in lodging resistance from its dwarf habit, and increased strength of culm base. In fact MI-273(m) showed these characteristics most markedly particularly in having as large a panicle but more tillers than H4. Leaf characters too are important in determining not only early plant development, but also during the period of grain filling. Only MI-273(m) maintained relative to H4 the area of the final three leaves. The flag leaf areas of the other mutants were reduced especially in K8(M) selection (K8(M)S). While K8(M)S is the shortest variety it lodges badly because the culms are weaker at the base. The diameter of the culm base of K8(M)S was less than that of all the other mutant lines.

Figure 5

MEAN LENGTH OF INTERNODES & PANICLE OF H4 & IT'S MUTANT DERIVATIVES



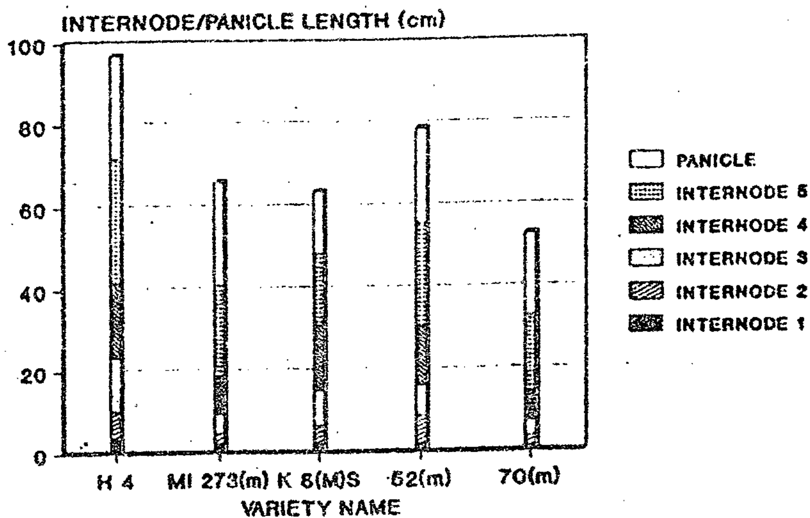
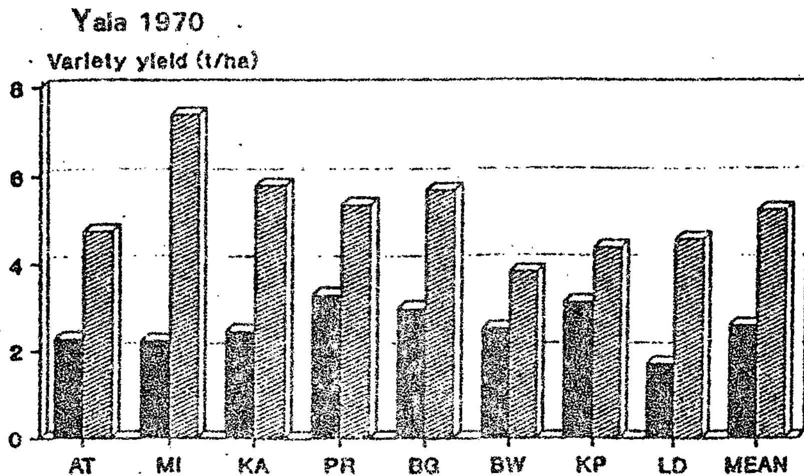
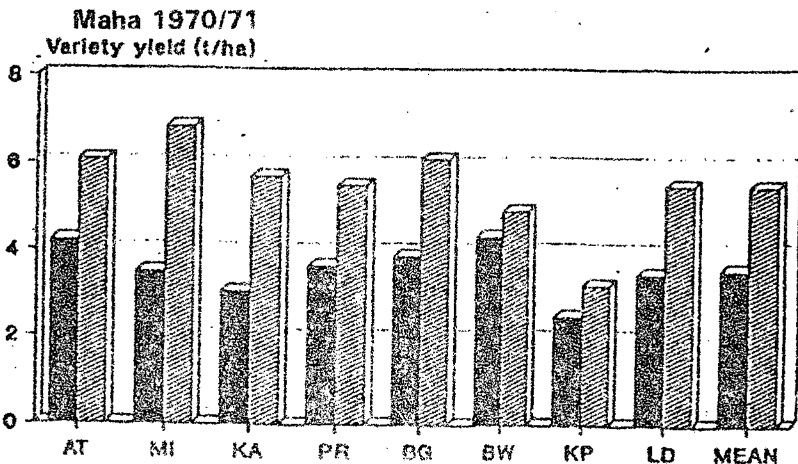


Figure 6

MEAN YIELD OF H4 & MI 273(m)
BY LOCATION





<u>DRY ZONE</u>
AT - Ambalantota
MI - Maha Illuppallama
KA - Karadian Aru
PR - Paranthan

<u>INTERMEDIATE ZONE</u>
BG - Batalagoda

<u>WET ZONE</u>
BW - Bombuwela
KP - Karapincha
LD - Labuduwa

K8(M)S shows similar changes in flowering time and in tiller number to the irradiated mutants, and while it might be argued that more than one genetic change could be induced by irradiation, it is less likely that K8(m)S, as a natural mutant, will be suffering from similar changes. It seems reasonable therefore to infer that alterations in the pattern of development from the original H4 are due to pleiotropic genetic effects.

Analysis of height differences in the F1 and F2 showed incomplete dominance for tallness in the H4 parent and evidence that four different loci with various degrees of dominance determined dwarfism (Ganashan and Whittington 1975).

Performance of MI-273(m)

a) Multilocational Varietal Trials

MI-273(m) was tested along with other promising varieties in the National Co-ordinated Rice Varietal Trials (NCRVT) at eight locations from Yala 1970 season. The grain yields and important agronomic characters from the **Maha** 70/71 and **Yala** 70 experiments where 12 varieties were continually tested over eight locations were analyzed for genotype X environment interaction effects by the method of Finlay and Wilkinson (1963).

This analysis was done on a seasonal basis and combination of seasons which shows the outstanding performance of MI-273(m) in each season by maintaining increased yield levels. In this study the semi-dwarf variety IR8, showed greater advantage in the **Yala** season than in the **Maha** season (Ganashan and Whittington 1976). Yield performance of H4 and MI-273(m) in the NCRVT at the eight locations during **Yala** 70 and **Maha** 70/71 seasons are given in Figures 6a and 6b. The increase in grain yield brought about in H4 by mutational changes, and the yielding stability of this variety and MI-273(m) in these locations and seasons are given in Figures 7.

Performance of MI-273(m) over large extents

To assess the potential of the important rice varieties they were grown in large extents rather in small experimental plots, where the management levels

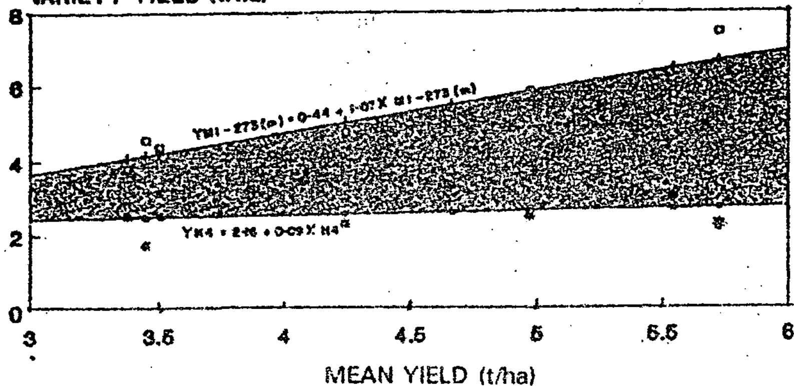
Table 3. SOME OF THE INDUCED RICE MUTANTS RELEASED FOR CULTIVATION

Name of the mutant	Country	Name of the parent	Year of release used	Mutagen used	Important characteristics
1. Jagannath	India	T-141	1969	X-rays	Medium slender grain, good cooking quality. Yield level 6 to 7 t/ha used in lowland coastal areas.
2. Dwarf H4 (former name MI - 273(m)	Sri Lanka	H4	1971	Gamma irradiation	Wider adaptability, potential yield 7.5 t/ha, larger number of panicles, panicle length unchanged. Erect growth, non lodging and non shattering habits. Can be harvested mechanically.
3. Yuan Fengzao	China	Kezi No.6	1975	Gamma irradiation	45 days earlier. 10% increase in yield, 8 to 14% higher lysine content.
4. Calrose - 76 (mutant D-7)	USA California	Calrose	1976	Gamma irradiation	25% reduction in height, panicle length unchanged.
5. RD6	Thailand	Khao Dawk Mali - 105	1977	Gamma irradiation	Glutinous endosperm resistant to blast, popular in Northern Thailand.
6. Kashmir Basmati	Pakistan	Basmati - 370	1977	Gamma irradiation	Early maturity, higher yield potential, aromatic, used in Northern Pakistan.
7. Miyuki - mochi	Japan	Toynishiki	1979	Gamma irradiation	Glutinous endosperm, other characters same as parent.

Figure 7

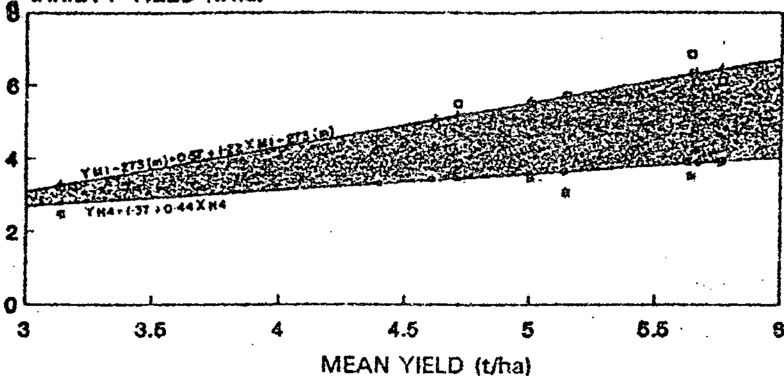
INCREASE IN GRAIN YIELD BY USING INDUCED MUTANT

YALA 1970



MAHA 1970/71

VARIETY YIELD (t/ha)



H4 (estimate)   MI273 (m) (estimate)

H4 (actual)   MI273 (m) (actual)

increase in grain yield

were not necessarily high. At Hingurakgoda Government Farm, Sri Lanka, under large-scale cultivation in **Maha** 70/71, MI-273(m) gave 100% yield increase over its parent variety H4, and outyielded all other varieties cultivated including IR-8(Peiris *et al*, 1972).

MI-273(m) was released for general cultivation in 1972. The first induced high yielding rice mutant released as a national registered variety was "Reimei" in Japan in 1966 (Futsuhara *et al* 1967). The yield increase in MI-273(m) over the parent variety H4 was very spectacular compared to "Reimei" and its parent variety, demonstrating the effectiveness of the mutation breeding programme carried out in Sri Lanka. Table 3 gives some of the important induced rice mutants released for cultivation in different countries.

MI-273(m) is preferred by farmers in the Northern, Uva and Sabaragamuwa Provinces and its adjoining areas. It is popularly used by farmers in the cropping pattern in the Badulla District in the Uva Province. It is also popular in the "**Kekulan**" areas of Vavuniya and Jaffna Districts, where occasional drought incidences are experienced. In 1973 farmers in the Omanthai area of Vavuniya District grew MI-273(m) and other popular BG varieties. These crops suffered a drought period of about 23 days at their panicle development stage. The drought incidence was so severe that the land in the cropped area started cracking and the crops were severely affected. When rains came after this 23 days of drought, only the variety MI-273(m) resumed its growth and gave yield of 3.25 t/ha; the other varieties failed completely (Pasupathy 1974). Thus where irrigation is not assured or rainfall is inadequate and where moderate management levels are practised, MI-273(m) is preferred. Recently the demand for the cultivation of MI-273(m) is increasing in the Sabaragamuwa region.

The rice variety H4 has been grown by Sri Lankan farmers for the past four decades, and perhaps it will remain in cultivation for decades to come. Wide adaptability, tolerance to environmental stresses, appreciable yield under low to moderate input conditions, and consumer preference had come from its parental variety Murungakayan 302 which was popular among farmers, and retained by them for long periods. H4 dwarf - MI-273(m) - forms an improved version of H4, and continues to be retained by local farmers.

Some farmers of the Uva and Northern region are maintaining the mutant MI-273(m) under the name "Short H4" (Kota H4 locally, "Kota" in Sinhala Language means shortness). They prefer the name short H4 to MI-273(m), because of their long standing preference to H4 variety, and its rice quality with red pericarp. Considering the farmers' preferences, the Department of Agriculture (DOA) has renamed this mutant as H4 dwarf in 1982 (Research Highlights 12, DOA, 1983).

Mechanized Harvesting of MI-273(m)

Heavy demands on labour at rice harvesting times force the cultivators to take to mechanized harvesting. Mechanized harvesting tests on MI-273(m), Bg 34-8, Bg 11-11 and 62-355 were carried out by the Farm Mechanization Research Centre, Maha Illuppallama, Sri Lanka in Maha 73/74 and Yala 74. The crops were raised at Maha Illuppallama by row seeding and by broadcasting under mudland condition, with high management practices.

Shibaura Reaper Harvester attached on small two-wheel tractor was used to harvest all the varieties at maturity. In this test MI-273(m) performed excellently compared to the other varieties which suffered shattering losses - Figure 8 (Pullainayagam 1974). In further tests, when the MI-273(m) crop had passed the harvesting stage (with 16% moisture content in the grains) negligible shattering losses of less than 0.05% were observed, because of its sturdy non-lodging culms and non-shattering grains (Pullainayagam 1974). Model Nanyue 4G Harvester from Peoples Republic of China, and the IRRI harvester from the Philippines were also tested for mechanized harvesting of MI-273(m). This mutant is continuing to remain as the leading variety suitable for mechanical harvesting even at present.

Induced Mutation Studies with *Brachiaria brizantha* Stapf

Brachiaria brizantha is the best adapted pasture grass for the dry zone of Sri Lanka. Prostrate growth habit, elongated internodes, and the presence of pubescence are some of the undesirable characters of this pasture grass, which have to be rectified by a breeding programme. An attempt made at RARC, Maha Illuppallama, in 1966 to improve this grass by hybridizing with other pasture grasses was not successful. Consequently, mutation breeding techniques were attempted to remedy these defects.

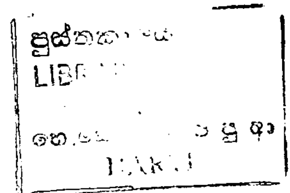


TABLE 4
EFFECT OF IRRADIATION ON SEEDS

Irradiation treatment (kR)	Germination percentage	Survival percentage of seedlings	Percentage of chlorophyll mutants
0	56	100	0
7.5	52	96	0
15.0	48	80	4
30.0	44	60	7
45.0	42	46	5
60.0	18	0	-
75.0	0	-	-

TABLE 5
EFFECT OF IRRADIATION ON STEM CUTTINGS

Irradiation treatment (k R)	Percentage of established cuttings	Initial growth
0	100	Normal
7.5	75	Normal
15.0	100	Retarded growth, narrow leaves, lightgreen streaks
22.5	75	Retarded growth, lean leaves, reduced pubescence
30.0	50	Retarded growth, reduced pubescence
37.5	40	Severely retarded growth, reduced pubescence and increase in lithality
45.0	40	Severely retarded growth and increase in lethality

Levels of Radiation Used

Seeds and stem cuttings of *Brachiaria brizantha* were subjected to 60Co gamma irradiation. The doses (kR) were as follows:

Seeds	1) 0, 2) 7.5, 3) 15.0, 4) 30.0, 5) 45.0, 6) 60.0, 7) 75.0
Stem Cuttings	1) 0 , 2) 7.5, 3) 15.0, 4) 22.5, 5) 30.0, 6) 37.5, 7) 45.0

Non-imbibed seeds were used in the seed irradiation treatment. For stem irradiation fresh cuttings of equal size, containing 3 nodes per cutting were taken from a well studied clonal selection.

Results

The germination percentage, percentage survival of seedlings and percentage of chlorophyll mutants are given in Table 4. Table 5 gives percentage survival of cuttings.

There was only slight reduction in the number of seedlings at the lowest dose of irradiation but at the higher dosage levels seedling numbers were greatly reduced. Chlorophyll mutants of the *albina* and *chlorina* types were observed at 15 kR, 30 kR and 45 kR treatments. Other types of morphological changes were also observed at 30kR, and 45kR treatments.

Stem cuttings subjected to radiation doses above 15 kR showed variations in leaf size and growth habit together with different types of chimaeras.

One mutant from the 45 kR seed treatment had an erect growth habit with short internodes, narrow leaves and reduced pubescence. This appeared to be the most promising mutant and was isolated for further study. Its chromosome number at pollen mother cell stage was 18, similar to the parent. It had a faster regrowth after defoliation, and a higher protein content than its parent.

Its erect plant habit would permit growth of an associated legume, while the lower pubescence would improve its palatability. The rapid rate of recovery after defoliation would permit grazing at much earlier stage of growth (Fernando and Ganashan 1967).

Development of Tissue Culture Activities in the Department of Agriculture

a) Rapid Clonal Propagation

The tissue culture technique was pioneered in 1976 by the Department of Agriculture (DOA) for the rapid clonal propagation of orchid and anthurium for cut-flower industry. This tissue culture activity is being continued at the Royal Botanical Gardens for commercial purposes. About 170 indigenous orchids of the Dendrobium and Vanda species are found in Sri Lanka.

In 1984, a Tissue Culture Laboratory was established at the Central Agricultural Research Institute (CARI), Gannoruwa, with the assistance of FAO for the pathogen-free micropropagation of fruit crops such as pineapple, citrus, banana, passion, and papaya. Since 1985 mass *in vitro* propagation of these crops was undertaken to raise homogenous plants for adaptability studies. In addition, micropropagation of other crops, such as **rambuttan**, strawberry, ginger etc., were also undertaken. Mini-tuber production and *in vitro* conservation of potato varieties were also done.

Micropropagation of potato through apical meristem culture to obtain disease-free plants was initiated at Regional Agricultural Research Center, Bandarawela, in 1987 and perfected in 1988. The technique is being used for mass propagation and distribution of disease-free stem cuttings to farmers for their own seed production programme since 1989. In this technology the meristem cultured plantlets are first tested for virus and those disease-free plantlets are grown on sterilized soil medium in aphid proof net-house to produce mother plants. Stem cuttings from these mother plants are planted in either sterilized soil beds to produce tuberlets - the basic seed - for further multiplication up to foundation seed, or the stem cuttings are directly issued to farmers for production of seed through one generation only before planting for consumption potato production. The mini tubers obtained from the mother

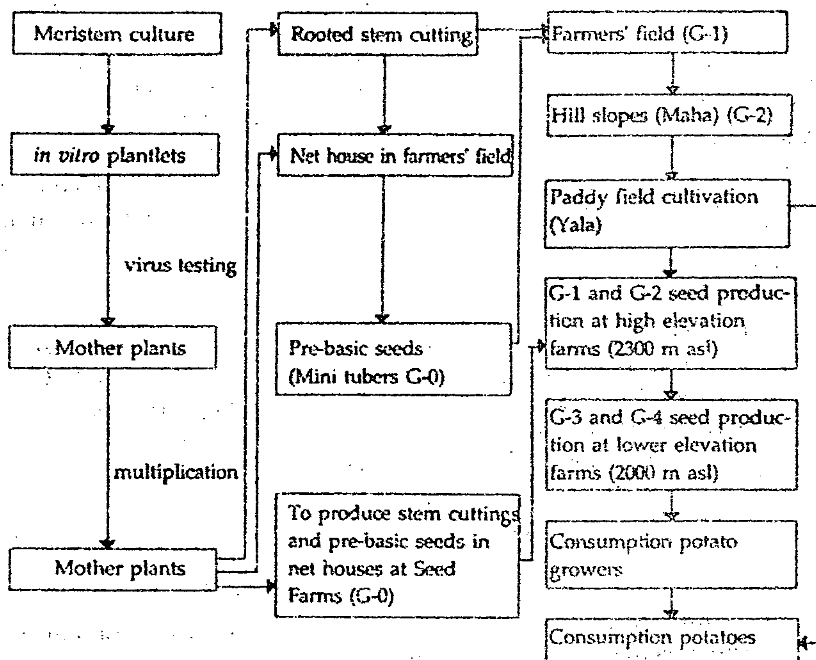


FIG. 9 SCHEMATIC SEQUENCE REPRESENTING MICROPROPAGATION OF POTATO AT RARC BANDARAWELA LEADING TO THE PRODUCTION OF FOUNDATION AND CERTIFIED SEEDS, INVOLVING DOA FARMS AND SEED PRODUCERS.

plant after the required quantity of stem cuttings have been obtained, are also used to produce basic seed (Figure 9).

This technology has benefitted the farmers in that they could now produce their seed requirement on their own and this could result in a large reduction in the imports of seed potatoes. Action is also being taken by the DOA to extend this technology into the upcountry Government Farms so as to produce "high grade" seed within the country, so that the seed potato industry will be independent of imported seed potatoes. Search for somaclonal variation in plantlets is also being carried out in the micropropagation programme (Yogaratnam, Personal Communication 1990).

The Plant Genetic Resources Centre (PGRC) which was established with the assistance of the Japan International Corporation Agency (JICA) initiated activities in 1988. It has facilities to undertake biotechnological research. *In vitro* conservation to maintain the germplasm resources of root and tuber crops such as sweet potato, cassava, potato, diascorea etc. are done at this Centre. Callus culture and plant regeneration studies in rice, embryo culture in groundnut, chilli, and rice, and electrophoretic studies in root and tuber crops are also being undertaken.

Potential Contribution of Biotechnology Involving Mutagenesis to Crop Improvement in Sri Lanka

Although advances in crop improvements have been made by using conventional plant breeding methods, there are limitations for further improvements. Some of the major constraints are given here along with the biotechnological methods which could be used to enhance the efficiency of traditional crop improvement methods, or where conventional methods have failed.

The germplasm derived by *in vitro* techniques needs significant testing at the whole-plant level. Plant breeding skills are necessary whether new variants arise from molecular, cellular or sexual manipulations.

Rice

Rapid adoption of new varieties for cultivation has resulted in narrowing genetic diversity in the field. The varieties currently in use have

genes predominantly from Cina and Latisail, both contributing from 18.8 to 50.0% nuclear gene sources (Javier *et al* 1989). All the new improved semi-dwarf varieties in use have their dwarfing gene from Dee-geo-woo-gen. In 6 popular rice varieties under cultivation, genes for gall midge resistance (vertical in nature), were obtained from OB 677/678. Five years after release, the gall midge resistance in these varieties and the donor OB 677/678 broke down. For brown planthopper resistance, PTB 33 formed the gene source; this also now shows a degree of vulnerability. Instances of breaking down of the resistance to blast disease have been reported. The narrow genetic base in the cultivated varieties has generated the risk of genetic vulnerability towards pests, diseases, and other hazards. In the absence of other donor varieties as parents, promising mutants available at present could be tried, or suitable programmes in mutagenesis initiated.

Currently mutation breeding programmes in rice are undertaken for cold tolerance at Pussellawa Research Station, and for photoperiod sensitivity, reduction in plant height, and resistance to blast disease at RARC Bombuwela.

Breeding for tolerance to such environmental stresses as salt, drought, adverse soil conditions, etc., also faces several difficulties. Plant biotechnologies offer plant breeders a mechanism for speeding up the development of varieties resistant to environmental stresses. Developing anther culture techniques and performing plant regeneration from callus offer efficient methods for the production of haploids. Callus induction and plant regeneration in *indica* rice is significantly lower than in *japonica* varieties. However, appreciable anther callus production of some Sri Lankan Bg varieties were reported by Pathinayake and Johnson (1989). Anther culture has the potential of compressing breeding cycles, increasing selection efficiency, providing for early expression of recessive genes, and exposing gametoclonal variants.

Potato

Potato is cultivated predominantly in the upcountry intermediate and wet zones, (7,000 ha) during the **Maha** and **Yala** seasons. However, a sizable extent (500 ha) is cultivated during the **Maha** season in some parts of the lowcountry dry zone (Jaffna and Kalpitiya areas) where the minimum temperature is around 22^{0}C. At this temperature tuberization takes place in some varieties. The minimum temperature requirement for tuberization is

considered to be below 22^{0}C. As some varieties tuberize at 22^{0}C in the dry zone, further improvement in developing types which could tuberize in both **Maha** and **Yala** season could be explored. Varieties which tuberize above 22^{0}C are not available elsewhere. Current potato production in Sri Lanka is threatened with severe attack of cyst nematode in the upcountry wet zone, which has led to greatly reduced cultivation. *In vitro* mutagenesis in potato protoplast and regenerations appears to be easier (Jones *et al* 1984), and this technique should be explored to develop varieties adaptable to the dry zone, so that potato cultivation could be increased.

Pigeonpea

The remarkable adaptability of pigeonpea varieties in the environmental continuum ranging from the drought-prone dry zone to the more stable environments in the intermediate zones has been observed. Pod borer (Maruca) is a serious problem and to overcome this, wild relatives as Atylosia and Rhynchosia species should be taken for hybrid embryo production, or even somatic hybridization could be tried. Wild relatives of pigeonpea are found growing under natural condition in Sri Lanka.

Induction of mutations at the haploid level, coupled with *in vitro* selection could form an important approach to develop pod borer resistant lines.

Groundnut

There is an urgent need to have a very short-aged and drought-resistant groundnut variety for cultivation in the dry zone. *In vitro* selection of induced mutations at cellular level should be tried.

Some of the immediate application of new technologies for the improvement of crop plants are: Induction and selection of useful mutants at cellular level is the most promising approach for developing varieties for stress conditions such as tolerance to salinity, drought etc., and for resistance to diseases when particular toxins are directly involved in the disease development. Anther culture should be taken up in species in which advances are made in pollen culture and plant regeneration.

The following goals for crop improvement using biotechnological

approaches involving mutagenesis should be taken up either locally or in collaboration with international institutes:

* Develop plant varieties that are tolerant to high salinity or flooding.

* Improve water use efficiency of plants and develop drought-resistant varieties.

* Improve the resistance of plants to diseases and pests.

* Modify the amino-acid composition of storage protein in cereals and legumes to improve nutritional value.

* Improve the composition and storage life of fruits and vegetables.

Fig.8 Mechanical harvesting of H 4 dwarf (MI-273 (m))

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TISSUE CULTURE OF TEA

P.V. Arulpragasam, B.Agr, M.Sc, Ph.D.
Tea Research Institute, Talawakele

Plant Tissue Culture occupies only a small niche in the vast field Biotechnology, but nevertheless is of great importance in agricultural development, the potential of which has not been utilized in developing countries like Sri Lanka.

The Necessity

The question arises as to why we should embark on a project of this nature when there in already a very successful and efficient method for the clonal propagation of tea. It is true that the present system of generative propagation has proved to be very useful and efficient, but it has its limitations as well and cannot cope with the present day needs.

The need for rapid and large-scale propagation of quality clones introduced in recent years has been reflected in our replanting and infilling programmes, in which estates still continue to plant with only a few of the long established clones because of the shortage of planting material of the newly introduced clones. Tissue culture techniques can be used to rapidly increase planting material of any clone, no matter how it was developed - whether by selection, breeding, genetic engineering or protoplast fusion. The rate of multiplication that could be achieved by this technique makes possible the evaluation and introduction of any new clone in much shorter time than is otherwise possible. By the conventional method it will take 12-15 years before mother bushes are established on a large enough scale to supply the needs of the Industry.

Our primary objective was to investigate the possibility of the propagation of tea by tissue culture and to develop a protocol for its successful implementation. The ultimate objective, however, is crop improvement. The current emphasis is to develop a strong technological base for plant regeneration from tissues, meristems, cells, anthers, embryos and possibly protoplasts plant regeneration is the fundamental, essential technology for any applications in crop improvement and production (propagation). Applying tissue culture and plant regeneration procedures in producing genetic variants for disease, pest and stress resistance will be some of the basic needs.

Progress Made in the Tissue Culture of Tea

Rapid, large-scale propagation using tissue culture techniques have been adopted for a wide range of plants. However, in the genus *Camellia* propagation by tissue culture techniques has had only a limited amount of success and still less in the case of tea, *Camellia sinensis*, the Queen of Camellias.

Plant tissue culture is the growth or maintenance of plant cells, tissues, organs or whole plants *in vitro*. Of the above *in vitro* systems we have been working on the regeneration of plants from:

a) Organs - Shoot tips, nodal explants and embryos

b) Tissues - **de novo** formation of organ primodia from callus tissues of cotyledons, stems and leaves or somatic embryogenesis

Each system utilizes the steps of explant establishment, propagule multiplication, plantlet regeneration and acclimatization.

The first system is the most widely used system in micropropagation. The main advantages of this technique is the increased likelihood of producing phenotypically uniform and genetically stable plants. In our work we used shoot tips and nodal segments with axillary buds as our explants.

Embryos obtained from mature seeds were also grown and multiplied in culture using this system, since this offers and easy and rapid way of

multiplying the few seeds that may be obtained by breeding.

The second system induces callus formation in tissues like cotyledons, stems or leaves. Plants could then be regenerated from the callus by inducing organogenesis or embryogenesis. Variations in the plants regenerated from callus tissues can be used for the selection of new lines.

Culture of Short Tips and Nodal Explants

Shoot tips and nodal segments were collected from actively growing plants like the plants recovering from pruning. Browning of the explants was prevented by dipping them in a 1.5% solution of PVP for 30 minutes and contamination was prevented to a large extent by first sterilizing in 95% ethanol for 45 seconds followed by sterilizing in 0.2% mercuric chloride for 20 minutes. This was followed by rinsing in sterile distilled water before explanting.

The establishment media contained MS[1] salts at half strength, thiamin, myo-inositol, and ascorbic acid. Asorbic acid was used as an anti-oxident and as a vitamin. The sucrose concentration was 3%. Cytokinin was used at 2.0 mg 1-1 and auxin (IBA[2]) was used at 0.1 mg 1-1. The cultures were incubated at 20-23^{0} C at a light intensity of 2000 lux with 16 hour photoperiod.

When the explants begin to proliferate in the establishment medium (6 to 8 weeks) they are transferred to the proliferation medium, which consisted of MS salts at full strength, increased cytokinin (BAP[3] 2.5 mg 1^{-1}) and reduced auxin (IBA, 0.01 mg 1^{-1}). Increased BAP concentration was found to enhance shoot proliferation and the decreased auxin concentration resulted in reduced callusing at the cut end of the explants. Two to three shoots were produced in the proliferation medium after six weeks, which were dissected asceptically and transferred to fresh proliferation media. Larger leaves were trimmed during

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[1] MS - Murashige and Skooge [2] IBA - Indol Butric Acid

[3] BAP - Benthyl Amino Purine

transfers. This process of dissecting out and transferring was carried out every six weeks. By using this technique it was possible to obtain about 610 plantlets within a period of one year form a single explant.

This technique of proliferation was found to be very efficient, reliable and reproducible for the large scale production of shoots in culture.

Rooting of the Shoots Produced in Culture

Microcuttings of shoots produced in culture were transferred to rooting media, which consisted of MS salts at half strength and low concentrations of auxin. Cytokinin was completely omitted from the rooting medium. Roots were initiated within one month in the rooting media. Rooting, however, was not consistent and it was not possible to obtain a large number of rooted plantlets. Studies on rooting using different pre-treatments, liquid and solid media, different concentrations of sucrose, calcium and other elements are being carried out.

Acclimatization

The rooted plants, once they have reached a height of 5-8 cm, were washed free of the nutrient medium and agar and were transferred to small plastic pots filled with fumigated soil and kept in the humid chamber for a period of one week to ten days. They were then gradually acclimatized to glasshouse conditions and then planted out in large pots and then in the field.

Embryo Culture

Embryos were obtained from nature seeds produced in the biclonal seed garden. The seeds were surface-sterilized by flaming after dipping in 70% ethanol. Embryos from these ungerminated seeds were aseptically dissected out and cultured in an agitated liquid medium. Small plantless with two to three unfurled leaves and with a small tap root were produced within three to four weeks. The plantlets were then transferred to solid medium and when sufficient growth had taken place, transferred to soil and acclimatized as described earlier.

Shoot tips and nodal segments taken from these embryo plants were

also multiplied as earlier. There was no contamination in the explants obtained from these plants.

Cotyledon Callus Culture

Pieces of cotyledons obtained from mature sterilized seeds were initially cultured in an agitated liquid medium. Increase in size of these explants were observed within ten days and callus formation was obtained after 2-3 weeks, after which they were transferred to a solid callusing medium and incubated in the dark for further callus formation. When sufficient amount of callus formation has taken place they were dissected out and subcultured further.

Good proliferation of the callus was obtained in the medium which contained 2, 4-D, within 6-8 weeks when incubated in the dark. When transferred to fresh callusing medium and incubated in the light the calli became firm. Embryoid like structures developed when transferred to a medium containing BAP as the only growth regulator. Some of the embryo-like structures turned green, some of which produced shoots. Some of the calli also produced embryos which were detached and germinated to small plantlets. The shoots developed from the callus tissue developed roots when transferred to another medium with less sucrose and without any hormones. The plants regenerated from these callus cultures were acclimatized as before.

Stem and Leaf Callus Cultures

Basic information is lacking on the regeneration of plants from stem and leaf callus tissues of tea. This information is important because some good clones are non-responsive to propagation by shoot tip culture.

Nodal segments, surface sterilized as described earlier, were used as explants for the induction of stem callus and leaf squares obtained from leaves produced in culture were used for leaf callus studies.

Initially only a rim of callus was formed around the cut end of the nodal segment and about eight weeks later these turned to globular structures which were hard and non-friable. Further proliferation was obtained by transferring to media with a high concentration of sucrose, kinetin and 2, 4-D.

Leaf explants started to form callus after six weeks at the cut ends and at the margins. The texture of the leaf callus was different from that of the stem callus, being soft and friable and greenish yellow. Callus formation was found to be better when whole leaves were used, since browning at the cut ends retarded callus formation in some instances.

Although embryoid-like structures were formed, regeneration of plants did not occur in any of the media tested. Root formation was observed in some cases.

Possibilities

A reliable and reproducible method for the multiplication of shoots in culture has been developed. From the work carried out so far it would be clear that tea can be propagated by using tissue culture techniques. Once a method for the large scale rooting of the shoots produced in culture is developed, immense possibilities for the rapid and large-scale propagation of tea will become available.

Cotyledon callus culture has been found to lend itself to long-term culture. This feature could be used in the selection of new lines for stress and disease tolerance. For, the length of selection is an important criterion, since selection agents that are used allow for the preferential growth of variants. Several generation times are required for the few variants or mutant cells to become the dominant cell types in the population before attempts are made at regeneration. Thus cotyledon callus culture offers a useful system for screening and selecting plants for disease and stress tolerance, and for the development of new lines.

Future Prospects

Germplasm Preservation:

The commercially cultivated seedling tea is extremely heterogenous and consists of hybrids of various combinations. The vast wealth of genetic material is being gradually lost due to replanting with a few selected clones. Tissue culture could therefore be used for maintaining a large collection of the desired

genotypes. This technique would also help facilitate the exchange of genetic materials between countries.

Embryo Culture:

This technique could be used to secure the survival of many difficult crosses in our breeding programme with clones of known characteristics.

Anther Culture:

This approach has not been successfully carried out in tea, is a proven method for stabilizing the recombinant variety and the useful traits of the F1 hybrid plants. Plants obtained in this manner can be multiplied by tissue culture techniques.

Protoplast Culture:

Work has recently been initiated for the isolation and culture of protoplast. This technique offers exciting prospects for the development of new lines and for crop improvement.

CONCLUSIONS

The rapid propagation of elite tea clones now appears to be feasible using tissue culture techniques and this can be used for the commercial production of millions of plants annually for replanting low yielding seedling tea. This would give better prospects for this export oriented commodity in countries like Sri Lanka, which depend on tea as a major foreign exchange earner. Mass clonal propagation of tea will have a positive impact on the economy of countries with large tea growing areas.

For crop improvement in the agricultural sector, whether it be tea or any other crop, micropropagation is the final step necessary to rapidly multiply any new variety or clone, whether it is produced by the conventional methods or by the more sophisticated methods of genetic engineering or protoplast fusion.

SCREENING OF YEASTS FOR CONTINUOUS ALCOHOLIC FERMENTATION

U. Samarajeewa, B.Sc, Ph.D
Professor, Department of Food Science
and Technology, University of Peradeniya, Peradeniya.

Fermentation of sugar solutions to produce ethanol is perhaps the most ancient biotechnological process used by man. The fermentation is brought about by introducing yeasts to a sugar solution stored in containers and allowing it to ferment till the yeast activity ceases, indicating the maximum production of ethanol. This method of fermenting in batches is practiced both on industrial and domestic scales today.

Alcoholic fermentation could be carried out more efficiently and economically as a continuous process. For continuous fermentation, yeasts that are active at a given range of ethanol concentrations are needed.

In selecting yeasts for batch fermentations, the production of ethanol by a given organism is examined in relation to time. The resulting plot of ethanol production (P) vs. time (t) giving the highest yields as well as highest slope is used to select the most suitable organism. In a batch fermentation, ethanol production is limited either by the non-availability of sugar or due to inhibitory effects of ethanol produced by the organisms themselves. In a continuous fermentation the limiting factor is the rate of growth of the organisms.

The growth and activity of yeasts is governed by the physical and chemical characteristics of the fermenting medium. Studies on the types of micro organisms occurring during natural fermentation of coconut sap in collecting pots indicated dominance of certain organisms at the initial stages of fermentation and certain other organisms at the initial stages of fermentation.

There were yet others that appeared to survive for a while at intermediate stages of fermentation (Atputharajah *et al*, 1986a). These observations indicated the role of sugar and ethanol, individually or collectively, in limiting the activity of micro-organisms during fermentation. Thus the concentrations of sugars and ethanol present at any given stage of fermentation are important criteria in determining the rates of fermentation.

During alcoholic fermentation nearly 50% of the mass of sugar is lost as carbon dioxide as shown by the stoicheometric equation below:

$$C_6H_{12}O_6 \dashrightarrow 2C_2H_5OH + 2CO_2$$

180g 92g (51%) 88g (49%)

Measuring the loss of weight during fermentation is a reliable and simple means of following the rates of fermentation under experimental conditions.

Examination of 144 yeast cultures, isolated from fermenting coconut sap, showed a linear relationship between the loss in weight and ethanol production when sterile coconut sap was fermented using the pure yeast cultures (Atputharajah *et al.*, 1986b).

Theoretical Consideration

The relationship between ethanol production (P) and the loss of carbon dioxide could be described by the equation,

$$P = W \times M_e / M_c \text{ ---------------- (1)}$$

where, W = carbon dioxide lost (g L^{-1})

M_e = molecular weight of ethanol

M_c = molecular weight of carbon dioxide

Tabera (1985), using a computer calculation program developed by Marquardt (1963) described the curve for ethanol production in relation to culture time (P vs. t) by the following equation,

$$P = at / 1 + bt \text{ -------------(2)}$$

where, P = concentration of ethanol produced

t = culture time

a and b = characters specific for individual yeast strains

Differentiating (1) and eliminating t in (2) the rate of fermentation could be described by the following equation at any required concentration of ethanol (P).

$$dp/dt = (a - dP)^2 / a \text{ ---------(3)}$$

At zero time the value of dp/dt = a (g ethanol) L^{-1} h^{-1}.

From equation (1), the ethanol concentration reached at infinite time is a/b (g ethanol L^{-1}). Thus the values of a and b could be calculated using the data P and t.

A plot of dp/dt at different concentrations of P is useful in predicting the rate of fermentation of a culture at a required ethanol concentration. The curves could be used to compare the efficiency of yeasts for continuous fermentation.

Coconut Yeasts

Of 144 yeast culture from coconut sap tested, 50 showed close relationship with the theoretical curve described by the equation (2) at ethanol concentrations above 5.5%, when the experimental curves for P vs. t were prepared. The method was applicable in further selecting yeasts out of the 59 cultures for continuous fermentation.

The activity of the selected coconut yeast cultures were compared by calculating and plotting the curves (dp/dt vs. P) using an American PC model 8088 microcomputer. The yeasts exhibiting higher rates of fermentation at ethanol concentration ranges of 5.5 to 8.5% were found to be different from the yeasts exhibiting highest ethanol production (P vs. t) (Wijesinghe and Samarajeewa, 1988). The use of kinetic plots (dp/dt vs. P) thus provides a suitable method in selecting yeasts from coconut sap for continuous alcoholic fermentation.

Fermentation of Molasses

The following yeasts were examined using kinetic plots for their ability to ferment molasses at concentration of 10, 12.5%, 15% and 17.5% total sugars and PH 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 induced using acetic acid or sulphuric acid:

Bakers yeast (Lessafre, France)
Sauterns yeast (Unican Foods, U.K.)
Champagne yeast (Unican Foods, U.K.)

Two cultures isolated from fermenting **Kitul** (*Caryota urens*) treacle. Of the five yeasts, the bakers yeast exhibited the highest rate of ethanol fermentation at an initial total sugar concentration of 12.5% and ethanol concentration range of 2 to 7%. All yeasts showed reduction of activity at increased ethanol concentrations above 5%.

The highest rate of fermentation was observed at pH 4.5 to 5.0, when adjusted using sulphuric acid. In the molasses fermentation industry, a pH of 4.8 is used. No fermentation occurred below pH 5.5 when acetic acid was used. The acetate ions appear to be toxic to fermenting yeasts below pH 5.5.

In relation to sugar concentrations, the highest fermentation rate was observed at 17.5% total sugar with bakers yeast. At 5% ethanol concentration this rate was 50% more than the rate at 12.5% total sugars.

CONCLUSIONS

Yeasts isolated from coconut sap and the bakers yeast use presently for batch fermentation of molasses could be used efficiently in continuous alcoholic fermentations. In batch fermentations the use of molasses at a total sugar concentration of 17.5% may increase the rate of fermentation significantly compared with sugar concentration of 12.5% used presently in the industry.

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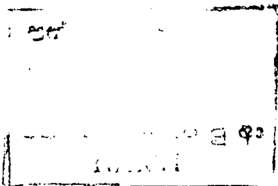

BIOTECHNOLOGY FOR PLANT GENETIC CONSERVATION AND BREEDING

P.K. Samarajeewa, M.Sc.
Plant Genetic Resources Centre
Department of Agriculture, Peradeniya

In agricultural crop production and for the sake of simplicity, biotechnology can be defined as "**manipulation of DNA, plant cell or plant parts in vitro to form a useful product or create a novel genotype**". For plant biotechnology, tissue culture or *in vitro* culture offers several important techniques applicable to genetic conservation and for creating new genotypes which are impossible with the conventional methods.

CONSERVATION OF GERMPLASM

Plant genetic resources are considered to become more and more important in future as they provide the main source of breeding materials for crop improvement. therefore conservation of genetic resources, which are being depleted fast due to various reasons have become more important. Today, world-wide attention is focussed on this goal.

Germplasm preservation is primarily accomplished in the form of seeds as they occupy a small space and can be stored easily for many years. However, this method cannot be applied to a variety of crop plants. These include vegetatively propagated crop plants such as banana, root and tuber crops. Certain other crops which mainly include fruit crop species such as avocado, mango etc. have recalcitrant seeds which loose viability when subjected to dessication and low temperatures and hence cannot be conserved in the same way as other seeds.

Though one can contemplate the field conservation of these crops the cost of maintaining them, especially annuals and biennials, in the field would be very high. Under field conditions they are prone to pests, diseases and other environmental hazards. In contrast to field conservation, maintenance of germplasm *in vitro* or in tissue culture form has the following advantages:

1. They are free of pests, diseases and other effect of adverse conditions.

2. Relatively little space is required.

3. *In vitro* propagation is rapid and can be accomplished irrespective of seasons.

4. Exchange of germplasm is easy.

IN VITRO CONSERVATION

As described by the International Board for Plant Genetic Resources (IBPGR) the activities related to *in vitro* conservation of germplasm are presented in Fig. 1. The germplasm received in any form is entered into *in vitro* cultures. For genetic conservation the use of organized tissues is of primary importance. Thus meristem - tips or shoot apices are considered to be the suitable starting materials for establishment of cultures. Occurrence of genetic variability in other tissue culture forms is quite evident (Bhojwani and Razdan, 1983) and hence they are unsuitable for germplasm conservation or clonal propagation.

The research conducted at the Plant Genetic Resources Centre (PGRC) has helped to develop *in vitro* techniques for several vegetatively propagated crop plants namely cassava, potato, sweet potato, yams, *colocasia*, **innala** and banana and presently a number of accessions in each crop are being held in *in vitro* form (Fig.2). In order to apply tissue culture for genetic conservation, techniques of meristem - tip or shoot - tip culture, micropropagation (Fig.3), plant regeneration, acclimatization and planting the tissue culture derived plantlets into soil have to be perfected for those crops.

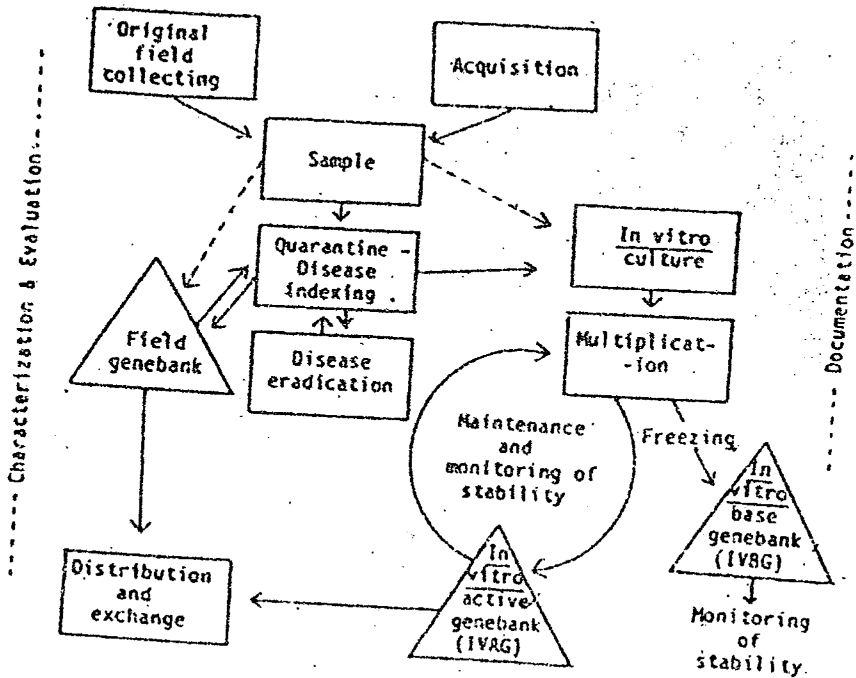

Fig. 1. In vitro genebank procedure.
(Excerpted from IBPGR report on Design, Planning and Operation of In vitro Genebanks.)

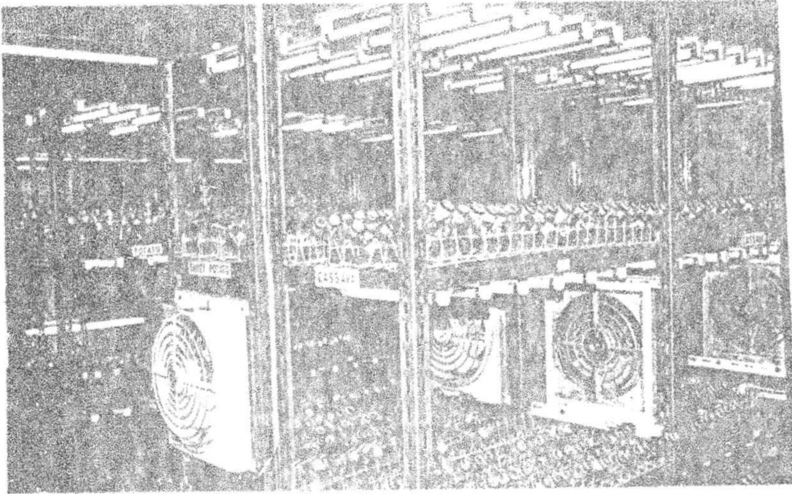

Fig. 2 In vitro storage of germplasm.

Fig. 3 In vitro propagation of *Gloriosa superba*.

Stored germplasm has to be free of disease especially of viruses and viroids. Therefore, wherever possible the germplasm is freed of diseases. For virus elimination the mother plants are subjected to thermotherapy at a temperature of 35-40°C for a suitable period prior to meristem-tip culture. The regenerated plantlets often are virus indexed employing one or more of the available methods, such as the use of index plants and ELISA technique.[1]

The cultures, growing under normal conditions, are subcultured at fixed intervals for their maintenance. Frequent subculturing however can result in loss of germplasm due to contamination and is uneconomical. In order to reduce the frequency of subculture two approaches are available.

1. Growth Retardation

This method is suitable for short-term or medium-term conservation of germplasm for several months to several years.

Growth retardation of cultures can be brought about in several ways:

i. Temperature Control

At low temperatures, culture period has been extended successfully for several crops. The experiments conducted at PGRC with cassava, sweet potato and banana have shown that the growth of culture can be reduced five fold by maintaining them at 15° C compared to those at 26° C. However, the temperature depends on the crop species: tropical species 15-20° C, subtropical species around 10° C, temperate crops 0-6° C.

ii. Light

Low light regimes are preferred for slow-growing cultures at low temperatures. However, some tropical crops like cassava seems to require slightly higher light intensities than other crops, like sweet potato.

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1 ELISA - Enzyme Linked Immuno Sorbent Assay.

iii. Culture Media Composition

a. adjusting the composition of inorganic nutrients in the culture medium,

b. varying the sugar concentration,

c. removal of cytokinin,

d. addition of mannitol or sorbitol.

2. Freeze Preservation (Cryopreservation)

This method can be employed for long-term conservation of germplasm (Kartha, 1985). Here the plant materials are frozen and maintained at the temperature of liquid nitrogen (-196° C). At this temperature the cells stay in a completely inactive state.

The procedures of freeze preservation involve the following major steps:

i. Pre-treatment of materials

This is done to enhance the freezing resistance of materials. In this procedure the material is treated with cryoprotectants such as dimethyl sulphoxide (DMSO).

ii. Preliminary freezing

The material is cooled at a temperature around -30° C to induc extra cellular freezing reducing the water content to prevent damage when cells are treated with liquid nitrogen.

iii. Freezing with liquid nitrogen at -196° C.

iv. Thawing: rapid thawing in water of 35-40° C is satisfactory.

v. Regeneration of plantlets

To regenerate plantlets from meristems or shoot apices the ordinary tissue culture methods are applied.

Studies have been carried out on the freeze preservation of a number of vegetatively propagated crops, including apple, cassava, potato and strawberry (Kartha 1985).

NEW GENOTYPES

Development of varieties through traditional methods which are based on sexual reproduction has been extremely successful. Despite that there is a constant demand for plant types, better adapted to their soil and climatic conditions, with built-in resistance to pests and diseases. The genes necessary to achieve those breeding objectives are often found in primitive cultivars and wild relatives. The attempts made through conventional methods to associate such characters with those of cultivated varieties have failed frequently due to various incompatibilities existing between them. Biotechnology helps overcome these limitations encountered in sexual breeding allowing greater utilization of a wide range of germplasm to produce novel genotypes. Further it provides opportunities to incorporate alien genes from nonplant sources such as micro-organisms into plants. Several important bio-technology techniques are discussed here.

a) Zygotic Embryo Culture

This is a more useful technique in tissue culture with immediate application to breeding by raising rare hybrids. In many interspecific and sometimes in intergeneric crosses, fertilization occurs normally and the embryo shows early development; however, poor or early development of endosperm results in premature death of the hybrid embryo. In such cases the embryos can be dissected out at early stages and cultured in a defined medium.

Embryo culture or embryo-callus culture has been successfully applied to produce many interspecific and intergeneric crosses. (Bhojwani &

for plant regeneration from protoplast culture, has increased considerably (Mendis, 1990).

Protoplast culture has two distinct applications. Cell fusion or somatic hybridization offers a completely new approach for distant hybridization especially where prefertilization in compatibilities are seen. The other most useful feature of plant protoplasts is that they can take up foreign DNA. These properties combined with the totipotent nature of plant cells have opened up avenues for creating new genotypes. However, no purpose is served by carrying out genetic transformation or somatic hybridization, unless a reliable plant regeneration procedure from protoplsts is available.

d) Anther and Pollen Culture

The discovery of haploid plant production from anther or pollen culture and doubling of their chromosomes to produce double haploids stimulated the interest of many workers since it seems to have several advantages in breeding:

i. A key problem in sexual breeding is the long period required to fix a new genotype. But in anther or pollen culture, a homozygous line can be produced in one generation saving considerable time in breeding.

ii. Complete homozygosity can be produced. This is difficult to achieve with sexual breeding alone.

iii. Haploid materials are useful for genetic studies.

iv. Haploid cells can be used for somatic hybridization

Anther or pollen culture techniques and conditions necessary for culture have been developed for a number of crop species and several anther culture derived new varieties have been released (Bollon and Requin, 1987).

CONCLUSION

In vitro preservation of germplasm by growth retardation is a simple technique which has been applied successful to several crops including cassava, potato and sweet potato. But with this method cultures cannot be maintained for a long period without sub-culturing. Though genetic variants *in vitro* culture of meristem or shoot tips have been discussed their occurrence has to be substantiated with more research. For long term conservation of germplasm, if the freeze preservation techniques are to be of practical use, further studies are needed to perfect methodology, with respect to each crop, enabling sufficient recovery of materials following freeze preservation.

With respect to application of plant biotechnology to genetic modification of plants, the techniques described here are mostly supplementary tools to conventional breeding. A genotype created through application of any of these techniques may have to be further manipulated with traditional methods to produce a desirable variety. Among these techniques, embryo culture is simple and can be considered to have an immediate impact on a number of hybridization programmes. It is also important to have the methodology of whole plant regeneration perfected from callus or cell suspension culture and protoplast cultures especially in view of the advancements made in the field of plant molecular biology and genetic transformation work. It may be concluded that the further development of plant biotechnology depends on the overall progress of biology and biotechnology cannot be considered as a separate area of research.

ACKNOWLEDGEMENTS

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THE CONTRIBUTION OF THE PRIVATE SECTOR TO THE DEVELOPMENT OF BIOTECHNOLOGY IN SRI LANKA

S.B. Rajakaruna, B.Sc. Agriculture
Group Agri-Environmental Manager
Ceylon Tobacco Co. Ltd., P.O. Box 62, Kandy.

Introduction

The Ceylon Tobacco Company (CTC) established its tissue culture loboratory in 1980, at Kalagedihena. This laboratory was equipped to produce approximately 200,000 plantlets; for the company nursery and other growers. Over time with CTC shifting its focus, as growers, from orchids to foliage plants, there arose an issue to orient the laboratory's services in the direction of contract with M/s Danefeldt Ltd., of Denmark and undertook to produce a limited range of tissue cultures provided by this company. Since the returns generated through this activity was inadequate CTC, in 1985, carried out a preliminary survey to identify the potential for elite planting material (food and other crops), in Sri Lanka. Accordingly, a project was launched to produce seed potatoes, as the survey revealed that there was a potential for seed potato, which could be developed through tissue culture. Simultaneously, CTC entered into a contract growing arrangement with Twyfords Ltd., U.K. and the laboratory facilities were elevated to produce one million plantlets per year. With the company entering into a contract with Sunki Pvt. Ltd., of Australia, again for contract growing there was an effort made to modernize the laboratory and the production capacity was then elevated to 1.6 million plants. In both these instances the outside companies provided the cultures and bought back the plantlets.

The CTC's experience in contract growing depending on imported cultures for commercial operations is as follows: high costs have to be endured to obtain cultures; uncertainty of markets; suppliers of these cultures, being

reluctant to purchase the plantlets developed with these cultures and lastly the decline in the global level markets for tissue cultured plants. This knowledge lead to CTC acknowledging that contract growing and total dependency on foreign markets cannot sustain the company in the long term. Therefore, the company turned its focus towards developing local markets for tissue cultured products. This resulted in the company directing its efforts towards research, development and production of elite planting material for the local market whilst still continuing with contract growing.

Current Programmes

Elite Planting Material for the Local Market

CTC has concentrated on the following:

a) Developing tissue culture and micro-propagation for the purpose of producing seed material of potato.

b) Producing high quality planting material for the fruit industry.

c) Tissue culture and propagation of ornamental plants.

Producing Seed Potato

Our objective in developing the tissue culture and micro propagation techniques for potato was to produce high quality seed tubers for the local market.

Potato is consumed as a vegetable in this country. The annual consumption is about 90,000 M/T. This is produced from 15,000 acres or 6,000 hectares of land. The amount of seed required to plant this extent annually is 15,000 M/T. This requirement is supplied as follows:

Farmers produce 10,000 M/T, through seed farmers and the balance 5,000 M/T are imported as certified seeds.

Our efforts are to replace a part or if not the total material imported, as certified seed, with seed material produced locally thus harnessing the tissue culture and micro propagation techniques that have been developed so far.

We have been successful in the development of tissue cultured plants for potato from disease indexed tubers. These **in vitro** material has been successfully planted out in the green house and through micro propagation have been able to produce zero generation tubers in growth tunnels, under insect proof conditions.

These tubers have successfully multiplied at the field level, and the first generation of certified seeds, has been obtained. Consequently, we are hoping to produce, within the next few years, sufficient quantities to the local needs of certified seeds thereby substituting for imports.

Planting Material for the Fruit Industry

In this area, we have been working mainly on banana, pineapple, grapes and passion fruit. In the case of banana, we have been successful in producing planting material through tissue culture of variety '**embul**' or 'sour banana', which has been tested for three generations whilst other varieties like '**kolikuttu**', '**unamalu**' and '**Williams**', cavendish variety, have also been successfully produced through tissue culture and are presently being evaluated.

Pineapple

Variety 'Mauritius' has been successfully multiplied through tissue culture and the plants are presently being tested out in the field. With regard to smooth Cayanne varieties, we are presently multiplying the variety 'Kew' which is revealing promising results. We intend to concentrate on this variety as it has a demand in the overseas market.

In grapes, our work is still in the experimental stage. We have been successful in the multiplication, rooting and hardening of the plants in the green house and a few plants have been tested out in the field.

Passion Fruit

It is worthy of mention here of our success with fruit crops. For the first time in the history of bio-technology, Passion fruit plants have been produced through tissue culture. This has been due to the research carried out by us, in our laboratory, for several years. This information has already been presented at the last session of the Sri Lanka Association for the Advancement of Science.

Ornamental Plants

Local varieties of Anthuriums have been successfully produced through tissue culture. However, we have so far not been able to produce plants of experiment varieties of Anthuriums, through tissue culture. Except for one imported variety, the other imported varieties of anthuriums have not responded to production through tissue culture.

The plantlets produced of local varieties of Anthuriums are being hardened in the green house and some have been transferred for field evaluation.

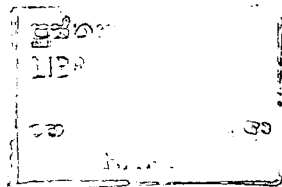
Successful attempts have been made in the propagation of foliage plants through tissue culture. Among the types propagated, are the Cordyline varieties 'Dracaena Warnackei', 'Song of Jamaica', which is a pleomela species, and Agloanema 'silver queen'. Here again, it is important to note that there has been little or no previous work done in the tissue culture of Agloanema. Therefore, our achievements Agloanema is noteworthy.

Future Directions

Our future plans are to explore local market opportunities whilst continuing with exports of contract grown material. We also, intend to build up a reputation as a reliable high quality low cost producer of elite planting material. In the interim, we are to develop an in-house R&D facility to work on new lines so as to gain a competitive advantage in the export market.

A programme, to evaluate the performance of tissue cultured material at the field level prior to it being released to the local market, is under way.

Lastly, we expect to establish a germplasm bank to store quality starter material, which will ensure an instant availability of the same to carry out production for the local and export markets.



IMPORTING PLANTING STOCK - THE IMPORTANCE OF PLANT QUARANTINE

Dr. S.N. De S. Seneviratne, B.Sc. Ph.D.
Formerly, Plant Pathologist, Central Agricultural Research Institute, Gannoruwa, Peradeniya.

The plants growing in a given country have not all originated there. Many have been introduced from elsewhere, a practice which has been going for centuries. In Sri Lanka, for example, many of the crops now cultivated here originated elsewhere: tea in Southern China, rubber in South America, coconut possibly in Central America, coffee in Ethiopia, cocoa in South America, pepper in India, banana in Peninsula Malaysia and Papua New Guinea and cassava in South America. Our staple-food provider, the rice plant, cultivated here for centuries, was itself an early acquisition which had originated in China or South East Asia.

In those distant times, planting material was freely moved from one country to another. There were no restrictions. But there were crop disasters from time to time. The fungus *Phytophthora infestants* devastated potato crops in Ireland in the 1840s contributing to the great famine which gripped that country in which about 1 1/2 million people died. And in our own country, another fungus, *Hameleia vastatrix*, caused the epidemics of leaf rust that led to the collapse of the nascent coffee industry in the 1860s, costing the country 17,000,000 sterling pounds.

It came to be realized in the course of time that while the benefits from plant introduction were substantial, there were dangers too: the entry of plant pests and pathogens with the planting material introduced; and that it would be prudent to adopt measures which while allowing the flow of the desired planting stock, would nevertheless control the hazards of associated pests and pathogens.

Plant quarantine had arisen as a defence reaction. In today's world, quarantine regulations of various sorts have to be complied with, phytosanitary certificates have to be obtained and, for the more effective operation of the controls, co-operation between countries has been developed - as with the Plant Protection Agreement for the Asia and Pacific Region which binds together more than 20 countries including Sri Lanka in a co-operative effort to prevent the introduction and spread within this Region of destructive plant pests and pathogens.

While those knowledgeable in matters relating to plant protection recognize the importance of plant quarantine, others regard it as irritating obstacle impeding the free importation of plant material. Those for whom such imports are commodities for sale generally care little about any adverse effects on agriculture and the environment in the long term. Quick profits are all that matter. What is distressing is the cynicism of some members of the scientific community, who cannot plead ignorance when they treat the provision of plant quarantine with scant respect. They often flout the provisions of the Act. There are also others who act above the law, the VIPs. Sometimes conscientious government officers lose favour over adverse reports by VIPs.

However, whatever the cynics may say and do, quarantine regulations and procedures are vitally necessary. They have been formulated to protect our agriculture and the environment from avoidable disasters, caused by hazardous organisms which may gain entry with introduced plant material and plant products, as well as with the packagings in which they are transported, or the soil in which rooted cuttings and plants are supplied.

Plants are subject to attack by pests and pathogens, of which there are many thousands the world over. In Sri Lanka too, the list of recorded pests and pathogens is a long one; yet, there are many dangerous pests and pathogens which do not occur here. Should they gain entry and get established, the consequences may well be catastrophic. Agriculture is the principal occupation of many millions in this country; agricultural commodities account for the major share of her export earnings; much of the food we consume is home grown. Any threat to Sri Lanka's economy and agricultural output by hazardous pests and pathogens that may be inadvertently introduced cannot be lightly dismissed. Adequate precautions must be taken to prevent such calamities.

Examples from rubber and rice can be used to emphasize the importance of plant quarantine in fulfilling our regional obligations and protecting our national interests. Rubber is an important plantation crop in Sri Lanka and other South East Asian countries. It is susceptible to a devastating disease, South East leaf blight, caused by the fungus *Microcyclus ulei,* which does not occur anywhere in our Region. Any lapses in preventing its entry will lead to disastrous consequences for the whole Region. Great strides forward have been made in increasing rice production from some 21.9 million bushels in 1952/53 to a record production of 127 million bushels in 1984. Part of this increased production is attributable to the fact that losses caused by diseases have been reduced to negligible proportions. The devastating disease, blast, caused by the fungus *Pyricularia Oryzae* affected many of the varieties grown earlier. Thus, Pachchaiperumal, which was very susceptible, had recorded crop losses of as much as 92%. This disease was overcome by incorporating a high degree of disease resistance into improved varieties that are now extensively cultivated. One of the most favoured donors of blast resistance used in breeding programmes Tetep, is highly resistant to the races of the blast fungus occurring in this country. But Tetep is attacked by races of the blast fungus occurring in Pakistan and the Phillippines. The entry of these races into this country will have catastrophic consequences. *P. oryzae* is seed-borne and their inadvertent introduction is a very real possibility. Indeed, it has already happened in Nigeria, Upper Volta and South Korea. In Upper Volta, for instance, rice yields which had been dramatically increased from 700 kg/ha to 10 tones/ha/year suffered a severe reverse when new strains of the blast fungus, able to attack the high yielding varieties cultivated, entered the country with seed introductions. Crops were severely affected and yields reduced to the miserable old level of 700 kg/ha. This should be a lesson for Sri Lanka. It will be wise to ensure that virulent strains of *P. oryzae* able to attack our varieties, are prevented, by the application of quarantine safeguards, from gaining entry into the country.

Crop improvement necessitates the important and introduction of germplasm from sources outside Sri Lanka. That is perfectly acceptable. What is not acceptable is that some introducers, ignoring the attendant risks, should circumvent the safeguards of plant quarantine in order to expedite their own crop improvement programmes. That these transgressors may be members of the local scientific community and may even be working for research institutions will not save them from defeating the very purpose of their efforts to improve crop production should their transgression result in the inadvertent introduction

and spread of some destructive plant pathogen. The outbreak of a severe epidemic of bacterial leaf blight caused by *Xanthomonas oryzae* at the Central Rice Breeding Station at Batalagoda in 1987 and the occurrence of virus disease at the International Winged Bean (Dambala) Institute at Pallekelle in 1986 both appear to have been caused by such improper introductions of germplasm - seed. These two cases may be elaborated on.

Bacterial leaf blight was recognized as a potential threat to rice production. The causal bacterium is seed and water borne. A large extent of paddy land received water from irrigation schemes. Accordingly, intensive screening of rice hybrids from breeding programmes was done with strains of this bacterium occurring in Sri Lanka and all the new improved varieties released were found to have adequate resistance to the disease. It was therefore surprising to hear of a serious epidemic of bacterial leaf blight affecting the improved variety Bg 94-1 at Batalagoda in 1987. *X. Oryzae* like he rice blast fungus, also has different strains. The Isabella strain of the bacterium in the Phillippines, for instance, attacks varieties resistant to other strains. Was the epidemic at Batalagoda caused by an alien strain of the pathogen which had gained entry with seeds directly introduced to our premier rice breeding station? I have long cautioned people in the Department of Agriculture that indiscreet introductions into station such as Batalagoda were to be fraught with danger. Whether they needed my advice is of course another matter.

There was also that famous International Winged Bean (Dambala) Institute which in its eagerness to bridge the protein gap of the country's undernourished millions, introduced seed of Dambala cultivars from various other countries with little consideration for precautions to avoid risk of disease. Predictably, what appeared to be a virus disease was observed in research plots at Pallekelle and I was commanded to investigate the problem. I complied. A virus was isolated from diseased Dambala plants, one which was able to infect many important leguminous crop plants cultivated here in Sri Lanka, including cowpea (cvs Arlington, MI 35, **Hawari Mae** and **Polom Mae**), green gram (cvs T 51, MI4 and T77) and black gram (cvs MI and T9), and also the leguminous crop plants such as **Vandaru Mae** (*Mucuna pruriens, Phaselous lunatus* and the common weed *Clitoria ternatea*. The virus resembled the *Psophocarus* ringspot mosaic virus which is prevalent in West Africa. It is probable that the virus was introduced to Pallekelle with imported seed. The introduction of a new virus able to infect other plants in Sri Lanka besides the

cultivated crops is a matter of serious concern and is a calamity that was avoidable had proper quarantine safeguards been applied.

Seeds, a frequent means of securing germplasm, also serve as carriers of many pathogens and pests, including viruses. About 90 viruses have been reported as seed-borne. Many accessions in germplasm collections worldwide appear to be virus infected. At the Western Regional Plant Introduction Station in Pullman, Washington, bean common mosaic virus (BCMV) was detected seed-borne in many of the *Phaselous* accessions in the germplasm collection and a strain of pea seed-borne mosaic virus (PSbMV) was detected in 38 out of 570 accessions in its lentil (*Lens culinaris*) germplams collection. At the Northeastern Regional Plant Introduction Station in Geneva, New York, pea seed-borne mosaic virus was detected in 420 out of 1835 accessions of ***Pisum sativum*** in the germplasm collection. In germplasm collections elsewhere, uridbean leaf crinkle virus has been detected seed-borne in green gram, bean common mosaic virus, mungbean mosaic virus and cucumber mosaic virus in mungbean; and uridbean leaf crinkle virus, bean common mosaic virus and broadbean mottle virus in black gram. It would appear that viruses are inadvertantly introduced with plant germplasm in the international transfer of genetic resources and it is therefore vital for us in Sri Lanka to strictly observe necessary quarantine safeguards so as to avoid reverses when working towards the objective of crop improvement and crop diversification with introduced germplasm.

Plant quarantine safeguard are of the utmost importance in preventing the introduction of insect pests too. Fortunately, most insects can be controlled by methyl bromide fumigation. However, with insect pests also, the practice of circumventing quarantine procedures has already caused near disasters, the culprits responsible usually being influential people and those with political clout. Some years ago, a very wealthy and influential person imported Mediterraanean oranges for his ailing wife. The Inspector of the Colombo Fumigatorium refused to release them because the laws of the land had to be observed. Outraged by the Inspectors's impertinence, this important person sought the intervention of VIPs to secure the release of the consignment. Fortunately, before that attempt succeeded, specimens of Mediterranean fruit fly had hatched out of the oranges. So Sri Lanka - Ceylon in those days - was spared the dire consequences of an entry of this illegal immigrant through the devotion to duty of a conscientious official. But another illegal immigrant, the coconut beetle,

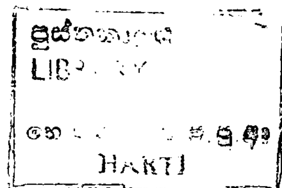
Promecotheca cummingii, which arrived in Sri Lanka from Singapore in the company of orchids, escaped to cause havoc in the coconut belt before it was eventually controlled. The orchids were apparently imported by a person who weilded considerable influences and political clout.

And now the destruction - perhaps it would be more correct to say, the sabotaging - of the flourishing potato growing industry of hill-country, with the introduction into Sri Lanka of the potato golden nematode also called the potato cyst nematode (*Globodera* spp. and *Heterodera* spp.). For the information of the uninitiated, it may be pointed out that this nematode is considered the most important of the pests of potato. Its microscopic eggs (also called cysts) have an excedingly tough shell and can remain alive in the soil for up to 30 years; they are highly resistant to desiccation and are easily dispersed; infestation with this pest could cause crop losses of 25-50%; land once infected must be laid off for 8-9 years before it can be planted again with potato.

In November 1984 cysts of this dangerous pest were detected in soil collected from crates in which a consignment of seed potatoes potatoes had been imported contravening quarantine regulations. Nevertheless, the Department of Agriculture had quickly cleared and distributed these seed potatoes. On 27th November, a group of the Department's specialists was asked urgently on how the situation which had arisen should be handled so as to avert the grave danger threatening Sri Lanka's potatoe cultivation. The group consisted of Dr. P. Shivanathan (Chief Plant Quarantine Officer), Dr. S.N. de S. Seneviratne (Plant Pathologist), Mr. I.D.R. Peries (Entomologist) and Mrs. H.M.R.K. Ekanayake (Nematologist) agricultural scientists who must be considered the most suitable persons in the Department to make recommendations in this matter. Apparently, the recommendations made by them were disregarded. Catastrophe for the industry inevitably followed. It is, of course, no secret that vested interests have manipulated for a long time in seed potato imports, distribution and sales to their advantage. The consequence of indefensible decisions that had been made have been borne by farmers who make their livelihood from the land they cultivate, the consumers and the country at large. Who is responsible?. Where does responsibility lie? These are some of the questions that must be raised and answered.

There can be no doubt about the importance of plant quarantine. Important also is the capability to execute the functions associated with plant

quarantine. This activity in Sri Lanka falls within the purview of the Department of Agriculture. More than 30 years ago, the Plant Pathologist at that time, Dr. J.W.L. Peiris, made proposals for the establishment of a plant quarantine station, Welisara which is close to the principal ports, being suggested as the preferred site. His advice was not heeded. Instead, attempts were made to site the station first near Rahangala, afterwards at Ambewela in the up-country. With the fortunes of the main actors changing, Gannoruwa became the favoured site. In due course, a station was established there. So plant material to be quarantined had to be transported many miles inland to an agricultural market place, bustling with people. A forward step was taken when an officer was assigned to take charge of plant quarantine, on a full time basis, whereas it previously had been 'overseen' by the Entomologist and Plant Pathologist. A very competent scientist was appointed Chief Plant Quarantine Officer. Unfortunately, he could not discharge his duties in the most desirable manner, weighed down as he was by administrative problems. In the matter of supporting staff, Plant Quarantine was treated as just one more unit of the Department to which officers, or rather 'bodies', were moved, not on their suitability for the work but on other criteria. Subordinate officers must submit, be flexible, malleable and adaptable in order to be acceptable to the hierarchy. And these "obedient servants" at the KIA have also to discharge a most important function - help clear the baggage of globe-trotting officials as they return with their acquisitions from their trips abroad. No organization with an important national service to perform can function that way. Plant Quarantine, if it is to be effective, must cease to be an appendage of the Department of Agriculture, and must be set up as a separate Department, staffed with competent people, provided with the necessary facilities, and sited not just anywhere but at a logically, scientifically, appropriate location. Above all, competent persons who know their job must be allowed to function effectively, uninhibited by the pressures applied by an uninformed administration.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THE NEEDS OF THE PRIVATE SECTOR FOR NEW PLANT GENOTYPES

R.L. de Silva, B.Sc, Ph.D, DIC
431/3, Havelock Road, Colombo 06

The title of this paper implies that the need of the private sector and those of the state sector (in respect of plant genotypes and indeed of other things) are different. This is precisely so. The private sector, while having their missions and corporate goals, have commercialism as their main objective. They endeavour to make profits by exploiting market opportunities and technologies under good management. If management is effete, the company will either go bankrupt or be subjected to mergers of acquisitions, often loosing their best staff in the process.

The objectives of the state sector are quite different. These could be national, political, social, financial or others. Enlightened governments do not usually wish to get involved in purely commercial activities unless under very special circumstanses. They do try, however, to manage incidental activities arising from their normal functions on a commercial basis such as marketing by-products from their research stations, or running the postal system. They prefer to leave commercial activity in the hands of the private sector which is especially equipped for handling commercial ventures.

In the agri-horticultural sector in Sri Lanka, apart from the Sri Lanka State Plantations Corporation (SLSPC) and the Janatha Estates Development Board (JEDB), the Government of Sri Lanka (GOSL) does not participate in purely commercial activity with the possible exception of some very small ventures. Even the SLSPC and the JEDB are soon to be privatised in stages. The state does, however, participate strongly in research and extension activities and to a limited extent in marketing agri-horticultural produce locally. With the

exception of the activities of the SLSPC and JEDB, the state is virtually absent from the export arena.

Requirements of Plant Genotypes for the Local Market

Plant genotypes are required to satisfy the needs of local consumers as well as to service industries which depend on plant raw material eg. the tourist and hotel industry, hospitals, schools, canneries, breweries, distilleries, the construction and furniture industries, and as a source of energy.

Both the state and the private sectors produce such plant raw materials, but with the exception of the timber and fuelwood raw materials, the private sector accounts for the bulk of the production. For the private sector to deliver the goods satisfactorily, it has to be emphasised that a most important aspect is security. Agri-horticultural produce needs outgoings in cash. The private sector will not make the necessary investments if there is no proper security for their produce against thieving and pilferage. Those caught thieving must be punished on conviction.

Catching them is difficult enough. Once caught, they cannot be allowed to get away on any account. If allowed it will kill the enthusiasm of the private sector and dry up investment in agri-horticulture. The larger establishments will take elaborate security measures, but this will only put up production costs.

Requirements of Plant Genotypes for the Export Market

The following are the important points to be noted:

a) Marketing opportunities must be seized promptly without loosing out to competitors.

b) The private sector needs assistance in research from the research institutes and the universities. The private sector in a country like Sri Lanka, cannot afford to set up their own research laboratories except on a very limited scale. They have to give priority to setting up quality control laboratories, and may not have the resources for elaborate research units. They depend on foreign technology. It is therefore, very

necessary that the universities gear themselves for carrying out contract research, as is done in developed countries and in many developing countries like India. The research institute too must gear themselves to doing contract research instead of merely depending entirely on government handouts from the tax payer. Such funds will be increasingly harder to come by. There has to be definite objectivity in research, with problems properly identified and the anticipated benefits from research clearly forecast. Reports of research stations should give the purpose of the studies, and the achievements, so that the reader is aware of the problem and its solution. In this way research studies can be more user oriented. The present system gives an appearance of weakness in research management and monitoring, and certainly implies under-utilization of resources.

c) There have to be clearly defined long-term plant improvement programmes, with specific responsibility for each species assigned to the organization held responsible for the development of that species. There have been instances of research being carried out in different laboratories on the same species merely because of personal interests. This is not a luxury that Sri Lanka can afford. The private sector needs the backing of the state sector in this long-term research programme for plant improvement.

d) There must be prompt action in releasing imported plant genotypes through the quarantine procedure. It is absolutely essential that importers be aware of the times at which they can be expected to receive clearance for the material they have imported. The time frame must be specified. No importer will be satisfied with an open-ended time frame. It is certain that marketing opportunities will be lost to competitors if quarantine procedures are long drawn out with vague time frames.

e) Again, security is an important factor. In addition to what was stated earlier, it is necessary for importers, growers, researchers and even the nation to protect their genotypes against unauthorised use. This is a difficult problem, but we must never loose sight of it.

Constraints Facing the Private Sector in the Procuring of New Genotypes

a) Plant Pathologists constantly over-emphasise the dangers of introducing new pests and diseases to the point of panic. While everyone will agree that the necessary rules must be enforced, there cannot be room for exceptions. In retrospect it would appear that the damage caused by the coffee rust, tea blight, Corynespora on rubber, Promecotheca on coconut, rice pests and diseases arose through deficiencies in screening procedures or in the use of cultivars or strains selected primarily for yield without adequate attention being paid to other criteria.

b) Research thrust appears to be unable to cope expeditiously in screening new genotypes, because the institutions are not equipped or oriented towards keeping up with the pace at which the new genotypes are needed.

c) Priority accorded for science and technology in Sri Lanka is low. It is said to be the lowest in South Asia (de Silva and Liyanage, 1987).

d) Sri Lankan exporters find it difficult to cope with competition form Australia, Indonesia, Malaysia, the Philippines, Singapore and Thailand, because those countries use highly sophisticated modern equipment in their work and can produce material the consumers demand, in a much shorter time than us.

e) Because of over-burdened teaching components, the universities can offer little help, to carry out research, in highly specialized fields, where the academic staff has expertise.

f) Lack of resources for research in the private sector impedes progress. Presently the private sector spends only about 7% of the total spent on agricultural research. This must be increased to about 50%, in view of the Governments, open economy policy and plans for the expansion of the private sector (Silva and Liyanage, 1987).

Finally, it must be remembered that agri-horticulture competes for private sector resources with a wide range of alternative investments. It is

REFERENCES

Liyanage, S., de Silva, M.A.T. 1987 (Report prepared for UNESCO). Sri Lanka Science & Technology Indicators (Part I) - Organisational structures & status of national efforts in science & technology. **NARESA**, Colombo, 93p.

Appendix I

Symposium on New Biotechnologies
ARTI: 14th June 1991
PROGRAMME

SCOPE

0845 - 0990 hrs -	Opening Remarks: Mr. Dixon Nilaweera Secretary, Ministry of Agricultural Development and Research
Session I -	Chair: Dr. O.S. Peries, Sri Lanka Standards Institution
0900 - 0930 hrs. -	Professor Ranjan Ramasamy Institute of Fundamental Studies, Kandy, "Biotechnology in the Year 2000"
0930 - 1030 hrs. -	Professor Eric Karunanayake Medical Faculty, University of Colombo "Gene Technology for Development"
1030 - 1045 hrs.	-------- TEA --------

APPLICATION

Session II -	Chair: Dr. R.L. Wickramasinghe Chairman, Sugar Cane Research Institute
1015 - 1115 hrs. -	Dr.(Ms) Shiroma Handunnetti Medical Faculty, University of Colombo "Research Towards Malaria Vaccine"
1115 - 1145 hrs. -	Dr. P. Ganashan Department of Agriculture, Peradeniya, "Genetic Improvement Through Induced Mutations"
1145 - 1215 hrs. -	Dr. P. Arulpragasam Tea Research Institute, Talawakele "Tissue Culture of Tea"

1215 - 1245 hrs. -	Professor Upali Samarajeewa Agriculture Faculty, University of Peradeniya "Screening of Yeast for Continuous Alcoholic Fermentation"
1245 - 1345 hrs. -	------- **LUNCH** --------
Session III -	Chair: Dr. P. Sivapalan Director, Tea Research Institute
1345 - 1415 hrs. -	Dr. P.K. Samarajeewa Plant Genetic Resource Centre, Peradeniya "Biotechnology for Plant Genetic Conservation and Breeding"
1415 - 1445 hrs. -	Mr. S.B. Rajakaruna Ceylon Tobacco Company Ltd. "The Contributions of the Private Sector to the Development of Biotechnology in Sri Lanka"
1445 - 1500 hrs. -	--------- **COFFEE** ---------

ALTERNATIVE METHODS - PLANT DISEASE PREVENTION

Session IV -	Chair: Mr. D.G.P. Seneviratne Director, Agrarian Research and Training Institute
1500 - 1530 hrs. -	Dr. S.N. de S. Seneviratne Plant Pathologist, "Importing Planting Stock: Importance of Plant Quarantine"
1530 - 1600 hrs. -	Dr. R.L. de Silva Formerly Director General, Sri Lanka Tea Board "The Needs of the Private Sector for New Plant Genotypes"
1600 - 1615 hrs. -	General Discussion
1615 - 1630 hrs. -	Summary and Conclusions: Dr. O.S. Peries Sri Lanka Standards Institution

List of Participants
Seminar on "New Biotechnologies"

P.D. Abayapala	-	Sri Lanka Environmental Federation
Gary Alex	-	US AID
N. Amarasuriya	-	CENWOR
P.V. Arulpragasam	-	Tea Research Institute
G.D. Bandara	-	Forest Research Centre
S.H. Charles	-	US AID
M.D. Dassanayake	-	Plant Genetic Resources Centre
S.C. Dharmaratne	-	Rubber Research Institute
S.C. Fernando	-	Coconut Research Institute
A.C. Gamage	-	Medical Faculty, Colombo
P. Ganashan	-	Department of Agriculture
R. Goonawardena	-	Medical Faculty, Colombo
Gemunu Gunegoda	-	Consumers and Users Federation
S.A. Handunnetti	-	Medical Faculty, Colombo
N. Iddamalgoda	-	Institute of Fundamental Studies
C.K. Jayasinghe	-	Rubber Research Institute
U. Kumaraswamy	-	Open University of Sri Lanka
R. Mahindapala	-	Coconut Research Institute
R. Nimalasiri	-	Lankadeepa
C.R. Panabokke	-	Agrarian Research and Training Institute
A.A. Lalith Perera	-	Coconut Research Institute
I. Phillips	-	Mahaweli Authority
S.B. Rajakaruna	-	Ceylon Tobacco Co. Ltd.
R. Ramasamy	-	Institute of Fundamental Studies
M.S. Ramasamy	-	Institute of Fundamental Studies
U. Samarajeewa	-	University of Peradeniya
P.K. Samarajeewa	-	Plant genetic Resources Centre
A.C.I. Smaranayake	-	Open University
L.K. Senaratne	-	Open University

P. Seneviratne	-	Rubber Research Institute
K.B. Shrestha	-	FAO
R.L. de Silva	-	Consultant
R.S.Y. de Silva	-	Consultant
P. Sivapalan	-	Tea Research Institute
D.B.R.Swarnathilake	-	Department of Export Agriculture
R.L. Wickramasinghe	-	Sugar Research Institute

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