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BIOTECHNOLOGY IN THE ESCWA MEMBER COUNTRIES SECTORAL ISSUES AND POLICIES

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PREFACE

Very recently, that is during the last decade, developments in the biological sciences culminated in the capacity to transform life. Futuristic scenarios with ever-green deserts, full of plants capable of shutting off their metabolism to withstand extreme environmental stress, are envisioned in the not too distant future. Unicellular organisms may be genetically changed and cultivated in large batches in order to mass produce rare food ingredients and pharmaceuticals. A cure for cancer may be closer thanks to advances in monoclonal antibody technology. In short, the promise of biotechnology is expected to exceed its present impact not only in the industrialized countries but all over the globe. It is not so much a question of whether developing countries will be affected but more a question of when this will take place and in what way.

To help the ESCWA member countries to better prepare themselves for the anticipated impact of biotechnologies, the present study provides an account of recent trends of direct interest to the region.

Biotechnologies are multidisciplinary in nature. Numerous scientific disciplines are involved in the manipulation of living cells and parts thereof. The successful development and application of biotechnologies thus requires careful coordination among many actors. More important, biotechnologies are multisectoral; equally affecting issues of interest to agriculture, industry, human health and the environment. Enormous benefits will be obtained for the economy, and the environment, as well as the well-being of humankind, if the necessary multisectoral and multidisciplinary approaches are adopted. Medical biotechnologies primarily serve to cut costs and save lives. The development of more specific diagnostics based on monoclonal antibodies is already turning into a generic technology in most industrialized countries. The early and precise detection of diseases, especially cancers, will allow early treatment and higher cure rates. As a result biotechnologies serving medical research have received more funding and attention in international research. As a direct spin-off of the international biotechnology industry is centred around this sector, many small specialized companies have emerged. Some of these companies have been successful to the extent of arousing the take-over instincts of big industrial conglomerates. This in turn has helped the biotech industry become a multibillion dollar business.

As the pharmaceutical industry is above all focused on satisfying the market needs of the high-income economies, diseases that are innate or more dominant in developing countries have tended to receive less attention. It is therefore in these countries that appropriate biotechnologies must be developed for combating diseases endemic to the developing world (such as leishmaniasis) and to some ESCWA member countries.

The food-processing industry is yet another sector which calls for region-specific biotechnologies. Fermentation of food crops such as dates and olives, which enjoy comparatively large markets in the ESCWA region, does not receive much international attention. Local research on bioreactors is therefore needed to support the development of new processes and improve the performance of more established ones for the benefit of the food industry in the region. Bioreactors also play a key role in the production of enzymes used in the beverages, detergents and leather industries. Although these sectors are well-developed in the ESCWA region, little research activity has been reported.

Despite the importance of the medical and industrial sectors, the present study puts most emphasis on agro-biotechnologies for two reasons. First, research on plants for crop improvement relates directly to specific ecological conditions predominant in the ESCWA region, whereas biotechnology applications to industry and human health are more universal in nature. Second, preliminary data indicate that most biotechnology research activities in the ESCWA member countries relate to agriculture.

In agriculture, the hunt for and manipulation of valuable genes is gaining momentum with the commercial cultivation of the first transgenic crops in China and the United States of America. The Fertile

Crescent, spanning parts of Jordan, Lebanon, the Syrian Arab Republic and Iraq, is where wheat and barley were first harvested. If the value of these resources is not acknowledged and action taken to preserve biodiversity, the consequences may be disastrous.

The technology to improve crops is mainly developed in industrialized countries, whereas the genetic input is provided by centres of genetic biodiversity which are mainly located in non-industrialized countries. For instance in the 1980s, American wheat and barley production suffered great losses, owing to an infestation of Russian aphids: no tolerant or resistant varieties of wheat or barley were available in the United States at that time. Only after incorporating genes from several Middle Eastern strains of wheat, using extensive crop-breeding programmes over many years, was it possible to manage the pest effectively (3). No country in Western Asia received compensation for the use of its germ plasm, but these same countries will have to pay for obtaining seeds of the improved pest-resistant varieties which incorporated genes of that same germ plasm.

Over the past few decades, agricultural production in Western Asia had to struggle to meet the needs of the rapidly growing population. Total arable land area in the Arab countries amounted to only 4% of the total area in 1994 (23). ESCWA member countries have become large importers of foodstuffs, mainly because of high population growth rates. An important factor limiting the increase in agricultural production is the critical and complicated problem of water supply and poor soil fertility in many parts of the region. Water problems are related to inadequate groundwater resources, erratic rainfall, water logging and salinity. Especially in the Gulf countries, owing to the depletion of groundwater resources, soil salinity imposes an increasing problem for crop cultivation.

Although the region is overall a heavy importer of agricultural produce and food commodities, the agricultural sector is given high priority in all government policies of the ESCWA members. The countries belonging to the Gulf Cooperation Council heavily subsidize both inputs and outputs in agricultural production. Because of this, Saudi Arabia recorded the world's strongest growth in agricultural production output in the 1980s and 1990s and has become a major food exporter (27). In 1994, the agricultural production index of Saudi Arabia had reached 506.55 compared with 100 in 1980 (26b). Other ESCWA member countries, such as Egypt and Yemen, are traditional agricultural countries with the majority of the population involved in cultivation of crops (Egypt: 33%; Yemen: 61% in 1994). The Governments of these two countries heavily support agriculture given their large numbers of rural poor.

PREFACE TABLE. NUMBER OF TECHNICAL PAPERS PRESENTED BY ESCWA MEMBER COUNTRIES AT THE ARAB CONFERENCES ON BIOTECHNOLOGIES ACCORDING TO SECTOR

Sector	First Arab Conference: 1989	Second Arab Conference: 1993	Total
Agriculture	6	9	15
Industry	4	6	10
Medicine	-	3	3

Source: ESCWA, Proceedings of the First Arab Conference on Perspectives of Modern Biotechnology in the Arab Countries (E/ESCWA/ID/89/15) (Amman, 1989); and Proceedings of the Second Arab Conference on Perspectives of Modern Biotechnology, R. Al-Natoor, ed. (San Diego, Bio Dynamics International, 1993).

Two Arab conferences on biotechnologies were organized by ESCWA in partnership with other United Nations organizations as well as the Higher Council for Science and Technology in Jordan in 1989 and 1993. They were the First and Second in Arab Conferences on Perspectives of Modern Biotechnology.

The results of both conferences showed that, as noted above, most biotechnology research in ESCWA member countries is geared to agriculture. Therefore, an analysis of agro-biotechnologies in selected ESCWA member countries will provide more insight to assist in analysing the problems faced in biotechnology transfer and development. As a direct follow-up of the recommendations of the second conference, the study investigates leading technologies (genetic engineering and plant cell and tissue culture) with the aim of clarifying the opportunities and challenges for the ESCWA region. To avoid generalizations the study allows for some differentiation in specifying policy recommendations for selected ESCWA member countries (i.e. Egypt, Jordan, Saudi Arabia and the Syrian Arab Republic) regarding the impact of biotechnologies.

Over the past decade, various Saudi Arabian public research institutions have undertaken the arduous task of developing the necessary elements for date palm micropropagation, including organogenesis protocol and facilities for large-scale handling of micro-propagated date palms. Despite these successful efforts, no joint collaboration materialized for further commercialization of the technology. Instead it required the input of technology from the United States of America to enable a private Saudi Arabian company to start commercial micropropagation of the date palm in 1996.

In the Syrian Arab Republic, as in the other ESCWA member countries surveyed, commercial opportunities for agrobiotechnologies are most likely to be found in the production of virus-free potato seed-tubers. Furthermore, research frameworks are in place, especially for cotton, to explore the potential offered by biotechnologies. To guide the expansion of micropropagation techniques to other crop commodities, the Syrian Arab Republic is needs specific policy formulation and implementation.

Biotechnologies are multisectoral in nature; furthermore, they increasingly blend in with other technologies such as reactor process technology and pharmaceutical production. Part two of this study highlights applications of biosciences in industry and medicine. Chapter V and VI comprise an overview of biotechnology activities in the ESCWA member countries surveyed.

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PART TWO
BIOTECHNOLOGIES FOR INDUSTRY AND MEDICINE

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ABBREVIATIONS

ABSP	Agricultural Biotechnology for Sustainable Productivity -United States of America
ACSAD	Arab Centre for the Study of Arid Zones and Dry Lands - Syrian Arab Republic
AFLP	Amplified Fragment Length polymorphism
AGERI	Agricultural Genetic Engineering Research Institute (Egypt)
ARCOMEX	Arab Company for Medical Diagnostics - Jordan
AUPAM	Arab Union of the Manufacturers of Pharmaceutical and Medical Appliances - Jordan
AECS	Atomic Energy Commission of Syria
ALECSO	Arab League Educational, Cultural and Scientific Organization
ARC	Agricultural Research Center - Egypt
ASRT	Academy for Scientific Research and Technology - Egypt
BAG	Biotechnology Advisory Group - Saudi Arabia
Bt	*Bacillus thuringiensis*
CARP	Center for Agricultural Research and Productivity - Jordan
cDNA	Complementary deoxyribonucleic acid
CGIAR	Consultative Group on International Agricultural Research
CIDA	Canadian International Development Agency
CSTR	Continuous Flow Stirred Tank Reactors
DAC	Development Assistance Committee
DNA	deoxyribonucleic acid
DPRC	Date Palm Research Center - Saudi Arabia
ESCAP	United Nations Economic and Social Commission for Asia and the Pacific.
ESCWA	United Nations Economic and Social Commission for Western Asia.
ELISA	Enzyme Linked Immunosorbent Assay
FAO-RNEA	Food and Agricultural Organization of the United Nations -Regional Office for the Near East
GATT	General Agreement on Tariffs and Trade
GE	Genetic Engineering
GLC	Gas Liquid Chromatography
GOSM	General Organization for Seed Multiplication - Syrian Arab Republic
GUS	ß-glucuronidase
GTZ	Gesellschaft für Technische Zusammenarbeit - Germany
HCST	Higher Council for Science and Technology - Jordan
ICARDA	International Centre for Agricultural Research in Dry Areas - Syrian Arab Republic
ICGEB	International Center for Genetic Engineering and Biotechnology
ILO	International Labour Organization
INOGE	Inter-Islamic Network on Genetic Engineering and Biotechnology
INRA	Institut National de la Recherche Agronomique - Morocco
JD	Jordanian dinar
JCVV	Jordan Center for Veterinary Vaccines
JICA	Japan International Cooperation Agency
JUST	Jordan University of Science and Technology - Jordan
KACST	King Abdulaziz City for Science and Technology - Saudi Arabia
KWH	Kilowatt hour
LE	Egyptian pound
MAbs	Monoclonal Antibodies
MIRCEN	Microbiological Resources Center
NAGEL	National Agricultural Genetic Engineering Laboratory - Egypt
NARI	National Agricultural Research Institutes
NARP	National Agricultural Research Project - Egypt

NARS	National Agricultural Research System
NRDC	National Research and Development Cooperation -United Kingdom
NBC	National Biosafety Committee - Egypt
NCARTT	National Center for Agricultural Research and Technology Transfer - Jordan
NRC	National Research Center - Egypt
ODA	Official Development Assistance
OECD	Organization for Economic Cooperation and Development
OPEC	Organization of Petroleum Exporting Countries
MEOR	Microbial Enhanced Oil Recovery
PCBs	Polychlorinated Biphenyls
PCR	Polymerase Chain Reaction
PCTC	Plant cell and tissue culture
PTC	Plant tissue culture
RAPD	Randomly Amplified Polymorphic DNAs
R&D	research and development
RFLP	Restriction Fragment Length Polymorphisms
RNA	ribonucleic acid
SAPAD	Saudi American Plant Development Co. - Saudi Arabia
SCP	Single cell proteins
SRLs	Saudi Arabian riyals
STC	Science and Technology Cooperation - USAID and ASRT - Egypt
UASB	Upflow Anaerobic Sludge Blanket Reactor
UNDP	United Nations Development Programme
UNEP	United Nations Environment Programme
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNIDO	United Nations Industrial Development Organization
USAID	United States Agency for International Development
WHO	World Health Organization

ABSTRACT

The contribution that biotechnology has made to improving agricultural production is generally associated with the achievements of genetic engineering. However, the use of plant tissue culture techniques appears to offer better solutions in terms of gestation period and potential for commercialization. Of course the choice of a technology should never supersede the identification of a research problem and the required interplay of actors which ought to allow for the adaptation and transfer of a technology. The present study highlights the importance of national policy formulation and implementation mechanisms to allow for optimization of alternatives offered by biotechnology. Part one of the study deals with agrobiotechnologies and part two focuses on biotechnologies for industry and medicine.

Part one also highlights technical and financial developments of the more advanced genetic engineering techniques in comparison with plant tissue culture, Chapter III surveys four ESCWA member countries (Egypt, Jordan, Saudi Arabia and the Syrian Arab Republic) which have developed certain capabilities in biotechnology. Chapters III and IV constitute the core of the study, which summarizes the status quo of biotechnologies in the selected countries. These chapters highlight some of the main differences between those countries and specify options for national policies with regard to technology transfer, development and adaptation.

In Egypt, biotechnology activities can be found in all sectors, including agriculture, industry and medicine. Private sector initiatives are, however, confined to the more generic techniques of plant tissue culture. Policies are not yet fully developed to serve as guidelines for the increasing number of promising biotechnology initiatives.

In Jordan the exception among the four surveyed countries, biotechnology policies are being developed and implemented. Several promising agricultural research initiatives are about to be implemented. However, more appealing incentives are needed to stimulate greater interest by the growing private sector.

Over the last decade, different Saudi Arabian public research institutions managed the arduous task of developing the necessary elements for date palm micro-propagation, including an organogenesis protocol and facilities for large-scale handling of micro-propagated date palms. Despite these successful efforts, no joint collaboration materialized for further commercialization of the technology. Instead it required the input of United States technology to enable a private Saudi Arabian company to start commercial micropropagation of date palm in 1996.

In the Syrian Arab Republic, as in the other surveyed ESCWA member countries, commercial opportunities for agrobiotechnologies are foremost to be found with the production of virus-free potato seed-tubers. Research frameworks are in place, especially for cotton, to explore the potential offered by biotechnologies. To guide the expansion of micropropagation techniques to other crop commodities, the Syrian Arab Republic badly needs specific policy formulation and implementation.

Biotechnologies are multisectoral in nature, and they increasingly blend in with other technologies such as reactor process technology and pharmaceutical production. Part two of the study highlights applications of biosciences in industry and medicine. An overview of prominent biotechnology activities in the ESCWA member countries is contained in the remaining chapters of the study.

PART ONE

GENETIC ENGINEERING OF PLANTS

I. GENETIC ENGINEERING OF PLANTS

Introduction

It is believed that the market for biotechnology-derived plant products will be valued at US$ 12 billion around the year 2000 (61a). Large multinational companies of industrialized countries are increasingly dominating the market. It is not just seed companies that have become involved in the strategic acquisition of small high-tech biotechnology companies: the chemical industry has also shown a keen interest in this technology over the years. The obvious intention behind the interest of the big industries is to link research in genetic engineering to their more traditional assortment of products, including pesticides and fertilizers.

Owing to the speed with which developments in biotechnology are taking place, the gap is widening between "biotechnologically developed regions" and regions where advanced biotechnologies have yet to be established. Industrialized countries have conducted more than 10 times as many field trials of transgenic crops since the first such experiment started in 1986 (see table 1).

TABLE 1. NUMBERS OF FIELD TRIALS ON TRANSGENIC CROPS IN INDUSTRIALIZED AND DEVELOPING COUNTRIES

Region	Number of field trials from 1986 - 1994
Industrialized countries	
North America	804
Europe	492
Asia*	53
TOTAL	1,349
Developing countries	
Africa	10
Asia	32
South and Central America	72
TOTAL	114

Source: A. F. Krattiger, "The field testing and commercialization of genetically modified plants: a review of worldwide data (1986 to 1993/94)" in Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere, *Genetic Engineering and Biotechnology Monitor*, vol. 2, No. 4, p. 4.

* Australia, Israel, Japan and New Zealand.

In addition, there has been a strong tendency for biotechnology firms, in the developed countries, to patent the results of their research, even when such results take the form of living entities or essential components thereof, e.g. gene sequences, proteins and chimeric organisms. An American company, for instance, is seeking to patent both partial and complete gene sequences, even where their respective biological activity has not yet been identified (31).

Investments in genetic engineering of plants are on the verge of bearing fruit, as the first transgenic crops are available in the market. Transgenic tobacco with increased virus resistance has been commercially cultivated in China since 1992 (4b). The first transgenic food produce, the "Flavr Savr" tomato variety with an improved taste and shelf-life, has been available on the American market since 1994 (12a). The transgenic

tomato was soon followed by genetically engineered maize in 1996, which has *Bacillus thuringiensis* genes, allowing the crop to produce its own pesticide (i.e. *B. thuringiensis* toxins) against the European corn borer. In August 1995, the United States Government approved the marketing of transgenic maize seed to take effect in 1996 (32). The marketing of a herbicide resistant soybean variety will also take effect in 1996 (64b). Table 2 contains an overview of commercial releases and field trials of transgenic plants.

TABLE 2. RELEASE OF TRANSGENIC PLANTS FOR RESEARCH FIELD TRIALS AND COMMERCIAL MARKETING IN OECD COUNTRIES

Crop species	Number of field trials	First field trial	First commercial release
Alfalfa	21	1988	-
Cantaloupe	14	1990	-
Corn	65	1990	1996 (32)
Cotton	37	1989	1996 (183)
Flax	49	1988	-
Rapeseed	290	1988	1995 (207)
Potato	133	1987	-
Soybean	40	1989	1996 (64b)
Squash	13	1990	1995 (12a)
Sugarbeet	28	1989	-
Tobacco	72	1986	1992 (4b)
Tomato	72	1987	1994 (12a)

Source: C. Brenner and J. Komen, *International Initiatives in Biotechnology for Developing Country Agriculture: Promises and Problems*, Technical Paper 100 (Paris, OECD Development Center, 1994), table 8.

Notes: Only plant species with at least 10 reported trials have been listed. References in parentheses in this table refer to the corresponding references cited in full in the section on "References" in this study.

Worldwide, China and the United States are the only two countries where large-scale releases of transgenic plants have been authorized for the purpose of commercialization (4a). In China, genetically modified virus-resistant tobacco has been used in industrial tobacco manufacturing for national consumption since 1992, and the cultivated area now stands at nearly 1 million ha or almost 5% of the tobacco planted area. In the United States, commercial production of genetically modified tomato started in February 1994.

Although the profitability of the transgenic tomato is still to be proved, virus-resistant, genetically modified tobacco yields an average of 5%-7% more leaves for processing and saves 2-3 insecticide applications out of a total of approximately 7 applications (4b).

The first mass-scale cultivation of transgenic cotton plants containing *Bacillus thuringiensis* genes took place in the summer of 1996 in the United States (183). Supposedly the transgenic cotton should be resistant to the cotton bollworm (*Heliothis zea*). Cotton losses caused by the bollworm are estimated at $216 million in the United States on a yearly basis (184). In the United States, the average cotton field undergoes 5 to 8 insecticide treatments per growing season. Unfortunately, weather conditions in the United States favoured a massive plague of bollworms, forcing farmers to rely on pesticides. It was in 1996 that the first crops of transgenic soybean and maize with resistance against herbicides were successfully harvested in the United States.

Genetic engineering of plants is currently one of the most advanced and promising biotechnologies. Internationally, the latest findings have resulted in protocols for single-gene transfer of an increasing number of plant species. Because of this, genetic engineering is becoming more important in breeding programmes for improvement of crops or novel applications. Applications of genetic engineering in crop improvement and the current status of related techniques as linked to approved field trials on transgenic plants are listed in table 3. From 1986 till 1994 a revolution in genetic engineering took place. In 1986 the first officially approved field trial of genetically transformed tobacco was conducted in the United States and France and in 1994 the first transgenic food crop (i.e. tomato) was released for commercial marketing.

TABLE 3. RELEASE OF TRANSGENIC PLANTS FOR RESEARCH FIELD TRIALS WITHIN DIFFERENT AREAS OF GENETIC ENGINEERING FOR CROP IMPROVEMENT

Application	Number of field trials	Percentage of total	Commercial release cases
Genetic markers for support of conventional crop breeding	382	30.4	n.a.
Installing herbicide resistance to crop species	489	38.9	3 Tobacco, maize, cotton
Installing resistance or tolerance against pests and diseases	239	19.0	3 Maize, cotton, squash
Improving characteristics of crop produce (e.g. shelf-life, flavour, texture, flower colour)	72	5.7	2 Tomato, rapeseed (13)
Installing tolerance to abiotic stress	12	0.9	n.a.
New products derived from crops (e.g. pharmaceuticals, organic solvents, plastics)	n.a.	n.a.	n.a.

Source: C. Brenner and J. Komen, *International Initiatives in Biotechnology for Developing Country Agriculture: Premises and Problems*, OECD Development Center, Technical Paper No. 100 (Paris, 1994), table 7.

A. RESEARCH DEVELOPMENT AND FIELD TRIALS

Genetic engineering is a unique technology allowing barriers between species to be traversed. This can offer many advantages for traditional crop breeding programmes, regarding the incorporation of novel traits, cost-effectiveness, speed and reliability (16a). Targets of genetic engineering of plants in industrialized countries are confined to added value of agricultural produce, resistance against plant pests using *B. thuringiensis*, or tolerance against particular licensed herbicides. Other achievements of genetic engineering include changing seed composition, flower colour, shape and vase life.

In 1991, the company Florigene B.V. of the Netherlands transferred a plant pigment gene (chalcone synthase gene) that changed the flower colour of the popular *Chrysanthemum* variety "Moneymaker" from pink to white. In Australia, Calgene Pacific Proprietary Ltd., Melbourne, successfully isolated the gene that controls the synthesis of the blue pigment delphinidin from petunia, which is now being transferred to rose with a view to producing a blue rose (195b).

The first transgenic crop with a modified seed composition—a lauric-oil rapeseed—was cultivated for commercial use in 1995 (13a). To date, rapeseed with modified oil content is the only example of a transgenic crop with a novel seed-quality trait that has been approved in the United States for unrestricted cultivation and commercialization. This has created an enormous research activity in creating new transgenic

rapeseed varieties which could lead to industrial products such as margarine, detergents, lubricants, inks, polymers, cosmetics and pharmaceuticals. Despite achievements over the past years, many growers and industrialists still have doubts about the immediate commercial future of oilseed biotechnology (13b).

The major concern is that most transgenic rapeseed varieties under development are substituted products of other oilseed crops or petrochemicals. This certainly holds true in the shorter term. Petrochemical resources will dominate the market for at least another 50 years. With the predicted inevitable depletion of non-renewable hydrocarbon reserves, it is clear that renewable plant oils will increasingly compete, in terms of price and quality, and could eventually be the only large-scale source of industrial hydrocarbons.

Another major concern is related to the cultivation of transgenic rapeseed crops. Although many of the new transgenic varieties may not progress to large-scale cultivation, there will still be a large number of transgenic rapeseed cultivars that are essentially identical in structure and growth habit, but that contain completely different seed products. This may cause formidable problems of seed segregation and identity preservation, particularly during cultivation, harvesting and crushing, in order to prevent cross-contamination of seed varieties (13c).

Most of the current field trials with transgenic plant material of more interest to developing countries that have been approved worldwide are focused on traits such as herbicide resistance (39%: see table 3), which in most cases could promote more intensive use of herbicides hazardous to the environment (44a, 24a). It is, therefore, environmentally more sound to focus on installing pest resistance to crops rather than tolerance towards herbicides.

This is the case with the gene transfer of *Bacillus thuringiensis* endotoxin genes. Different strains of *B. thuringiensis* produce protein toxins active against different groups of insects including the Lepidoptera (moths), Diptera (flies and mosquitos) and Coleoptera (beetles). The toxins accumulate as crystals inside the bacteria during sporulation. The toxins are believed to be biodegradable and inactive against mammals and other animals (45).

Using different gene transfer techniques (e.g. the *Agrobacterium tumefaciens* leaf disc transformation method, electroporation, gene bombardment) the *B. thuringiensis* genes can be inserted into many plant species of economic interest. The first field tests of transgenic cottons containing a *B. thuringiensis* gene were conducted in 1991 (25). To date *Bt* gene constructs have been genetically engineered into corn, cotton, potato, rice, tobacco and tomato (30a).

A cause of concern in using such technology is the increasing number of cases of insect resistance against these endotoxins. Researchers are therefore trying to incorporate *B. thuringiensis* genes jointly with a plant chitinase gene to prevent or delay the development of such resistance (32).

Entomologists, however, have voiced concern about the installation of *B. thuringiensis* endotoxin genes into plants (44b, 208, 33a, 30b). When plants excrete such endotoxins continuously and uniformly, some insects will soon develop resistance causing the loss of a valuable natural resource which is environmentally safe and effective for a broad range of insect pests. Moreover the traditional way in which the endotoxins are produced by fermentation of *B. thuringiensis* is straightforward. Simply spraying the crop at the right time will eradicate pests and, at the same time, limits overexposure of the endotoxins to insects. Annual sales in 1995 of *B. thuringiensis* strains and products have been estimated at US$ 125 million per year (30c) representing approximately 1% of the US$ 15 billion in pesticides marketed worldwide. Another disadvantage of *B. thuringiensis* products is their inconsistent performance in the field. This in turn has been reflected by the emergence and departure of several *B. thuringiensis* producers in the past 5 to 10 years (33b).

Another recently mastered application of genetic engineering of plants is the installing of virus resistance in plants. Viral diseases are generally untreatable as the virus has incorporated its DNA or RNA into the genome of the host plant. Transferring a single virus gene that encodes for the coat protein of the virus effectively protects plants against subsequent viral infection. Viral coat protein-mediated protection has been conferred to potato, cucumber, squash, melons, papaya, and legumes (57).

In 1991 the first transgenic crop field trial was conducted in Australia with potatoes resistant to the leaf roll virus (41a). The coat protein gene of the virus was installed into the plant by genetic transformation. Outside the plant cells the virus is packaged in its coat protein which it has to shed inside the plant in order to replicate. In the transgenic potato, the coat protein is present in abundance and will replace the coat so the virus cannot unpackage itself. In the beginning of 1995 the Upjohn and Asgrow seed company started selling transgenic squash seeds with resistance to watermelon mosaic virus 2 and zucchini yellow mosaic virus (12a).

The above-mentioned examples show that genetic engineering is rapidly gaining a foothold in most disciplines of agricultural research. The first applications are proving to be successful. However, the technology has yet to be fully integrated into standard crop breeding programmes. This would benefit crop breeders as well as the technology itself. The following section will explain in more detail which techniques resulted in the mastering of transferring genes from one species to another.

B. TECHNIQUES

For successfully transferring desired genes into plants, the following key technologies needed to be mastered:

(a) Isolation (cloning) and characterization of genes to be transferred (e.g. PCR, RFLP, RAPD, AFLP, microsatellite based markers);

(b) Construction of a vector containing a promoter, the gene to be transferred and a reporter gene (e.g. GUS, luciferase, nopaline synthase);

(c) Gene transfer technology (e.g. *A. tumefaciens* transformation system, electroporation, micro-injection, particle bombardment);

(d) Regeneration protocols for transformed plant cells or tissue.

The first two technologies were well established at the beginning of the 1990s (see table 4). The success of the latter two techniques (i.e., gene transfer technology and plant regeneration protocols) are currently the rate-limiting steps in establishing transgenic plants. Since genetic transformation of plants is dependent on a proper transformation system and regeneration of transgenic cells or tissue, it was not until 1986 that the first authorized field trial with genetically transformed tobacco could be conducted.

Monitoring the expression of inserted genes into bacteria such as *E. coli* was made possible in 1981 with the advent of Northern blotting,[1/] which allowed the blot transfer of mRNA (messenger RNA). The

[1/] Northern blotting amounts to separating fractions of RNA electrophoretically in a gel, transferring the RNA from the gel to a special paper. The now fixed RNA can hybridize with radioactively labelled probes for identification purposes.

construction of vectors, which could shuttle gene encoding for desired characteristics into plants, experienced a breakthrough in 1981 when the promoter[2/] of Cauli-Mosaic viruses was identified.

The systematic search and isolation of desired genes from higher eukaryotes was not possible until 1983 when RFLPs (Restriction Fragment Length Polymorphisms) were developed as genetic markers for eukaryotic genomes. Although this was becoming a routine operation, it was still a costly and time-consuming affair to locate and isolate genes of interest using RFLP maps. Genetic maps consisting of RFLP markers have been constructed for a variety of plants including barley (1992), maize (1991), wheat (1989), rice (1988), lettuce (1987) and tomato (1986) (53a).

TABLE 4. TIME FRAME OF DEVELOPMENTS OF DNA MANIPULATING TECHNIQUES LEADING TO ISOLATION AND CLONING OF GENES TO BE INCORPORATED INTO A VECTOR FOR GENETIC TRANSFORMATION

DNA manipulation technique	Year of first application	References
Transformation of *Escherichia coli*	1970	(50a)
Cloning of foreign DNA into a vector	1972	(50b)
Agarose gel electrophoresis	1972	(50c)
Southern blotting	1975	(50d)
DNA sequencing	1977	(50e)
Chemical synthesis of genes	1979	(50f)
Northern blotting	1979	(50g)
Caulimovirus vectors for plant transformation	1981	(50h)
Restriction Fragment Length Polymorphism (RFLP)	1983	(50i)
ß-glucuronidase reporter genes	1987	(52)
Polymerase Chain Reaction	1988	(51a)
Randomly Amplified Polymorphic DNAs (RAPD)	1991	(51b)
Amplified Fragment Length Polymorphism (AFLP) Microsatellite based Markers	1994	(54)

Note: References in parentheses in this table refer to the corresponding references cited in full in the section on "References" in this study.

In 1994 two new classes of genetic markers emerged to speed up the mapping of traits. AFLP (Amplified Fragment Length Polymorphism) markers are a combination of RFLPs amplified with the PCR which allow for the faster detection of a larger number of informative markers in certain plant species. Microsatellite-based markers, by accessing simple sequence repeats, are so far the most informative markers available (54).

In 1988, the polymerase chain reaction (PCR) allowing for the rapid multiplication of targeted DNA or RNA looked like a promising way to speed up this process. Only in 1991, however, could the PCR technique be used for identifying a more advanced class of genetic markers, the so-called Randomly Amplified Polymorphic DNAs (RAPD). The use of RAPD has the advantage of less time consumption, no

[2/] The promoter of a gene is a DNA sequence which is recognized by an enzyme which triggers the expression of genes.

use of radioactivity and a larger number of markers. One major disadvantage of RAPD markers, however, is the problem of not being able to provide simple as well as unique genetic markers for several plant lines (195a). This makes it difficult to correlate results obtained by different research groups (54).

Despite the discovery of many new methods for pinpointing genes, the isolation and characterization of genes is still an arduous task, with unpredictable time spans. Since the exact location of a gene on a chromosome is never known beforehand, the search for DNA sequences often resembles the well-known needle in a haystack, with millions of DNA nucleotides to sequence. The search is furthermore confused by many non-coding regions on chromosomes.

The Human Genome project, a joint undertaking of public and private research bodies all over the world, has generated a new technology useful in isolating new genes: the rapid-throughput automated cDNA (complementary DNA) sequencing. This allows for the determination of sequences of large portions of DNA in a relatively short time frame.

In 1981 the first expression of foreign genes in plant cells has been reported (50h), making use of viral vectors. Several other genetic transformation systems of plants have been developed, as listed in table 5.

TABLE 5. GENETIC TRANSFORMATION SYSTEMS OF PLANTS AND THEIR COMPARATIVE DISADVANTAGES

Transformation system	Disadvantages	First report
Viral vectors (194)	• Limited potential for the production of transgenic cereals. • Viruses do not integrate into host genome. • Excluded from meristems and thus from transmission to sexual offspring	1981 (50h)
A. tumefaciens (55a)	• Limited host range (monocotyledonous crops cannot be transformed). • Regeneration from tissue culture is a prerequisite. • Long time frame.	1983 (50j)
Direct DNA transfer (electroporation or chemical fusagens) (55b)	• Regeneration from protoplasts is a prerequisite.	1985 and 1987
Microinjection (55c)	• Regeneration from protoplasts is a prerequisite.	1985
Particle bombardment (55d)	• Low transformation frequency.	1987

Note: References in parentheses in this table refer to the corresponding references cited in full in the section on "References" in this study.

The most advanced and promising genetic transformation system to date is particle bombardment (55e). This technique allows for the transformation of organized tissue, thereby bypassing requirements for regeneration of the transgenetic material through somatic embryogenesis or other plant tissue culture techniques. This is especially beneficial for those crop species of which only tissue culture protocols for limited varieties (1 or 2) have been developed, such as maize, wheat and cotton. Moreover, particle bombardment is a variety-independent transformation system cancelling the need for extensive back-crossing programmes. Therefore, elite cultivars of crop species can be directly targeted for genetic transformation, resulting in faster cycle times in breeding programmes and field testing.

The American transnational company Du Pont has the exclusive rights to the technique, which is advertised as "easy to use and well within the capabilities of even a small laboratory" (199e). To insert a gene into specific crops is the easy part; the more arduous task is to make them work. An obvious disadvantage of particle bombardment is not knowing how and where the inserted DNA is incorporated into

the plant genome. A low transformation frequency is an additional drawback. Thus, to establish whether a transferred gene is securely incorporated into the recipient genome still requires extensive field testing programmes.

C. FINANCIAL ASPECTS

Rough estimates suggest that the development of a genetically modified plant costs about US$ 10 million, of which US$ 1 million is for regulatory expenses and requires approximately six years to go from the laboratory to a commercial product (61b). By comparison, traditional genetic breeding costs between US$ 2 million to US$ 2.5 million, and requires a 6- to 10-year development period (61c).

Obviously, genetic engineering of plants can only be profitable by focusing on improving those characteristics that will warrant investment, such as herbicide tolerance or high value-added tomatoes. Incorporating herbicide tolerance in crops is especially beneficial for the chemical industry, as pesticide development costs range between US$ 50 million and US$ 70 million (61d), of which 25% is for environmental and health testing. These high costs also warrant the interest of chemical companies in the development of pest-tolerant or pest-resistant plant species through conventional breeding programmes or genetic engineering.

An elegant projection of the potential economic impact of transgenic herbicide-resistant maize in the United States was provided by Loren Tauer in 1989 (48a). As is the case with most new technologies, consumers may benefit because there is a greater quantity of corn being produced at a lower price. Agricultural producers in the aggregate (including all crop and livestock producers), however, would experience a decrease in farm income. This decrease occurs because when farmers adopt this profitable technology, it increases the output of corn, causing prices to drop and farmers' incomes to decrease. The study indicated that only in those areas of the United States where weed is a significant problem for maize (causing 20% of crop losses) would corn producers benefit, even if corn prices fell. Possible commercial benefits for the agricultural research community are more difficult to project as there is severe competition between medical research groups to obtain grants.

Today, genetic engineering research activities are mainly confined to applications in medicine and agriculture. By comparison, research activities in medicine have received more attention from academic institutions, private enterprises, Governments and the public. The public and the Governments of most countries allocate high priority to health issues, which explains the large amounts of money spent for medical purposes and the big market size of pharmaceutical products.

The development of a pharmaceutical product requires investments of between US$ 300 million and US$ 400 million, with a time span of 12 years to reach the market (63). Therefore, medical research has been significantly better funded by both private investors and the United States federal government than basic research in the agricultural sciences (see box 1).

By comparison, plant scientists in the United States have also endured more hindrances from government regulations. Field research regulations, focused as they are on the use of the new genetic techniques rather than on tangible risks, have been estimated to increase the price of a field trial, on average, as much as 100 times that of a field trial with a similar, conventionally bred plant (57b). In addition, agricultural research must address fundamental questions in over 300 different species. On average competitive grants for plant biology have been 33% smaller and only 25%-50% as long as biomedical research grants.

BOX 1. BIOTECHNOLOGY RESEARCH AND DEVELOPMENT IN THE UNITED STATES

To indicate the commercial opportunities for biotechnologies and genetic engineering in particular, the experiences in the United States are a good example, as the United States is the world leader in biotechnology research as measured in scientific publications, the number of private enterprises and expenditure for research. The United States Government spent US$ 4.3 billion (17) on biotechnology research activities in 1994: the biotechnology industry accounted for US$ 7 billion in spending on research and development (64a). In 1994 a total of 1,311 biotechnology firms were active in the United States. The emergence of biotechnology companies in the country was characterized by two phases. The first started in the 1970s and lasted till 1987 when the first new biotechnology firms were established. These firms originated from research organizations, selling scientific and technological knowledge, but no products. The Government encouraged such private initiatives especially through the advanced technology programme, to provide federal funding to accelerate the development of promising but economically high-risk technologies that could enhance the general economic productivity. The Government also encouraged intimate collaboration between private companies and government-supported laboratories. Such technology transfer, in which the federal Government pays for a considerable part of the research costs, is seen as mutually beneficial, since it facilitates the sharing of expertise, personnel and material. The second phase, which started in the 1980s, was characterized by the integration of the new biotechnology firms with established firms. New biotechnology firms started to establish manufacturing facilities while established firms explored in-house biotechnology research and development. The main reason for this development was to avoid transaction costs. The agrobiotechnology industry in the country is comparatively small, covering only 8% of total sales in biotechnology products and services. There are only a small number of companies working on genetic engineering of plants and these are generally large, established agricultural firms. In sharp contrast, private R&D companies in the biomedical sector started as small new biotechnology firms. The technologies and products of the agrobiotechnology firms are, therefore, diffusing only slowly through the broader traditional commercial agriculture sector which includes plant breeders, farmers, food manufacturers, and retailers. The financing of research is a big problem, as most companies lack sufficient cash-flow owing to a negative return on investments and long gestation periods. The investment ratings in stock offerings are low. Standard & Poor's 500 index for agrobiotechnology companies has been below 100 since 1984, whereas the S&P 500 index of biopharmaceutical companies reached a peak of 350 in 1992 (57c,d). Furthermore, as many agrobiotechnology firms are getting close to marketing their products, there is a need for increased R&D spending for regulatory approval, field testing, and product introduction. Huttner therefore suggests that the American agrobiotechnology industry is in need of new kinds of public-private partnerships (57c). In the meantime, a new revolution of mergers seems apparent. In June 1995 Monsanto, the leading company active in agrobiotechnology, indicated its intention to acquire 49.9% of equity stakes of Calgene, the first company to sell genetically engineered tomatoes, in exchange for US$ 30 million and research collaboration (64b).

Plant scientists therefore have to allocate considerably more time into securing funding than do medical scientists. P.W. Simon, a plant geneticist of the University of Wisconsin, indicated that 50% of his working time went into fund-raising activities (49). Overall this has resulted in less fundamental knowledge of plant genetics as well as of biochemical and metabolic pathways of plants, which limits applications for the genetic engineering of plants. This is particularly the case for the manipulation of multi-genetically inherited traits.

Medical applications of genetic engineering are largely confined to one species (*Homo sapiens*) and focused on one product (health). Since the applications of genetic engineering of plants focus on more than one species and more than one product (including food, fibres, organic solvents, fuel, plastics, and medicines), there are more diverse investment opportunities in this field.

Equipment costs

Equipment costs for outfitting a basic plant genetic engineering laboratory are estimated to be at least US$ 600,000 (see table 6). Running costs of such a laboratory are very much dependent on the type of research, salaries of research personnel, cost of maintenance and supplies. For non-industrialized countries, salaries and basic supplies are considerably lower, whereas prices for equipment and advanced chemicals (e.g., restriction endonucleases, Taq polymerase, radio-isotopes, ELISA kits) can be more than twice as expensive as in industrialized countries (67). The lower level of maintenance in non-industrialized countries will reduce the using time of equipment, which in turn increases the running costs of the equipment.

TABLE 6. PRICES OF KEY EQUIPMENT FOR A GENETIC ENGINEERING LABORATORY

Equipment	Prices (1992, in US$) (68)	Prices (1996, in US$) (54)
Scintillation counter	146,000	-
DNA synthesizer	73,000	30,000
DNA sequentiator	73,000	70,000
Gamma counter	73,000	-
Radiation laboratory	73,000	-
Freezer (-70°C)	58,000	15,000
Photography equipment	29,000	20,000
Centrifuge with rotors	22,000	50,000
Gel electrophoresis systems	18,000	-
Computers	15,000	-
Particle Gene Bombardment Equipment (66)	12,000	-
Spectrophotometer	11,000	3,000
Biosafety hood	7,000	-
Balances	7,000	-
PCR equipment (67)	6,000	-
Eppendorf Centrifuge	4,000	-
TOTAL	627,000	549,000

Note: References in parentheses in this table refer to the corresponding references cited in full in the section on "References" in this study.

D. FUTURE DEVELOPMENTS

The current revolution in genetic engineering of plants is mainly due to gene transfer techniques whereby genes with desired characteristics can be inserted into a foreign plant species. The gene can be inserted into the recipient plant genome using different gene transfer techniques. All these techniques have a common feature: the location and frequency of insertion into the foreign genome cannot be determined. In addition, the number of copies of the particular gene, successfully incorporated into the genome, can only be controlled to a certain level. Therefore, only single gene-determined traits can be manipulated successfully. These single gene traits can include resistance against some biotic pests and diseases, herbicide resistance, control of the ripening of fruits, as well as starch and amino acid content.

Tolerance against abiotic stress forms a special challenge as these traits are multigenically determined. At present, directly manipulating a group of genes simultaneously is far more complicated and for the most part impossible with the present techniques of genetic engineering (14a). Complex multigenic traits are difficult to manipulate, because all of the specific genes that control the traits must be identified and inserted together in a transfer agent where they must function in the required sequence or in coordination, as necessary, to cause the beneficial change, an almost impossible task.

Today, scientists with the Rockefeller Foundation's International Program on Rice Biotechnology are trying to use genetic engineering of rice to make it drought-tolerant. So far, using conventional breeding combined with molecular markers has proved to be more successful in identifying and selecting those plants with the desired drought-tolerant traits (12b).

Single gene transfer experiments showed some promise, as in 1995 slightly increased drought tolerance was recorded, with transgenic tobacco containing the gene which encodes for an osmoprotectant. This method of overproducing osmoprotectants, however, is limited by the "cost" to the biochemical machinery of plant cells. Over-expression of the same gene in maize resulted in deformed kernels with little starch biosynthesis (56a).

Plant tolerance against aridity, salinity and low soil fertility are examples of such extremely complex multigenic traits, of specific interest to the ESCWA region. The first two characteristics, aridity and salinity tolerance, are related to capacities of plants to survive in an environment depleted of their most crucial ingredient for life: fresh water. Not only does this constitute the medium in which all bioreactions take place, but it also forms one of the two main ingredients of photophosphorylation, the reaction whereby water and carbon dioxide are turned into basic building blocks of carbohydrates. In other words, depletion of water implies that plants are denied access to both energy and water at the same time.

Therefore, plants tolerant of arid conditions have developed mechanisms to go into a state of dormancy and stop all processes which cost energy and prevent evapotranspiration. This concerns a cascade of biochemistry pathways (e.g. citric acid cycle, carbohydrate metabolism, lipid metabolism, amino acid metabolism, nucleotide metabolism). In fact all processes in plants are affected in cases of water shortage. The molecular genetics governing the metabolism processes are very complex. Control of gene expression in eukaryotes not only takes place at the transcriptional level, but also at the transnational and post-transnational levels. Presently there is little knowledge of transnational regulation taking place within eukaryotes (19), let alone the processes controlling the gene expression of an entire organism.

Plant tolerance of salinity requires an even more complex mechanism of genetic regulation, as salt-tolerant plants seem to be capable of discriminating between water with chemically distinct properties (20). If the salt concentration of water surrounding the roots is too high, the plant blocks uptake of water and relies on mechanisms of drought tolerance until water with lower salt content is available.

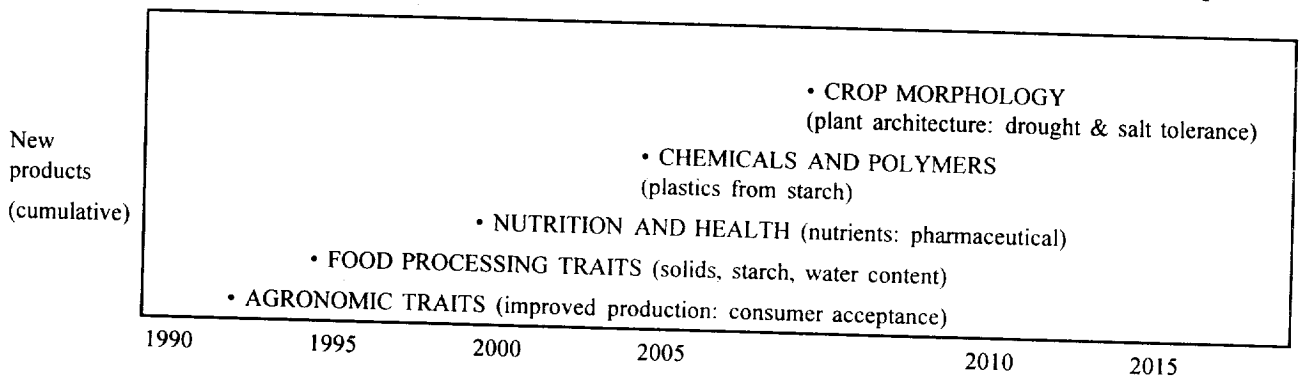
To sum up, although tolerance of abiotic stress is of prominent importance to the region, tangible results using genetic engineering techniques are not foreseen in the near future. Breakthroughs are expected with regard to changing multigenic inherited traits around the year 2015 (see figure I). The time frame for other expected application areas of genetic engineering of plants is shown in figure I. The entry of products for new application areas in the market-place is dependent on the rate of development of fundamental knowledge of the genetic and biochemical bases of the specific traits involved (57a).

Future prospects of genetic engineering of plants are dramatic and in time will create an impact on agriculture which might change the whole sector. Genetic engineering of plants is, however depicted as the

most complex biotechnology at present (58) (see figure II). However Sasson (5a) claims that genetic engineering of animals is more complex.

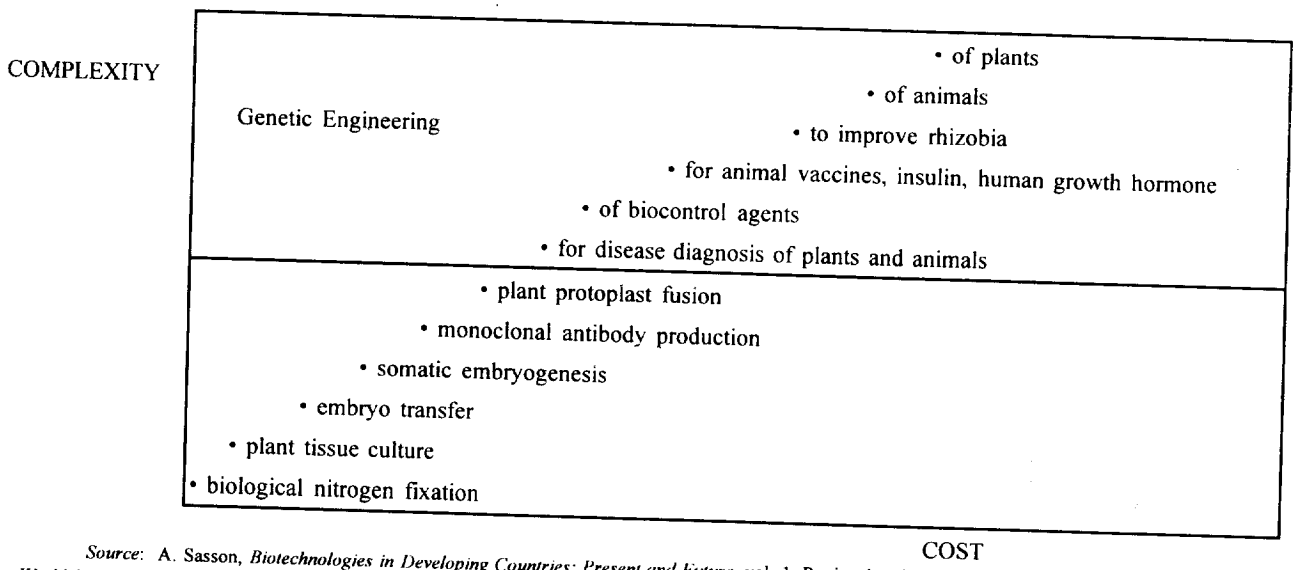
The first complete genetic engineering of animals was achieved in 1982, when Palmiter (50k) introduced a rat growth hormone into mice, which grew significantly larger. It was not until 1986 that the first field trials were conducted with genetically transformed tobacco. Because there are more ethical strings attached to genetic engineering of animals, it will take more time to ensure that the necessary legal regulations are in place to proceed with research in this field.

Figure I. Likely time course for the introduction of major biotechnology product groups



Source: S.L. Huttner, H.I. Miller and P. 6. Lemaux, *U.S. Agricultural Biotechnology: Status and Prospects*, Technological Forecasting and Social Change 50 (New York, Elsevier Science Inc., 1995), figure 1.

Figure II. The gradient of biotechnologies



Source: A. Sasson, *Biotechnologies in Developing Countries: Present and Future*, vol. 1: Regional and National Survey (Paris, UNESCO, 1993); and the World Bank, *Agricultural Biotechnology: The Next "Green Revolution?"*, World Bank Technical Paper 133 (Washington, D.C., 1991).

E. Developments in selected ESCWA member countries

Only some countries in the region have established facilities for molecular biology research (see table 7). Of those countries, Egypt and Kuwait have undertaken genetic engineering research for crop improvement; however, only Egypt succeeded in genetically transforming a plant by installing virus resistance into potato lines. So far only ICARDA (International Centre for Agricultural Research in Dry Areas) established the use of molecular biology as a supportive tool for plant breeding by developing genetic markers. There are no private initiatives in genetic engineering of plants reported in the region. The more promising Egyptian and ICARDA initiatives in molecular biology research will be described in more detail below.

Table 7. Institutes in ESCWA member countries involved in plant molecular biology research

(Numbers of institutes per country indicates the minimum number of national research institutions)

ESCWA member States	Number of institutes
Egypt	3 (69)
Iraq	1 (70)
Jordan	2 (67)
Kuwait	1 (5b)
Syrian Arab Republic	1 (71)
TOTAL	8

1. *Egypt*

The only research institute in Egypt is the Agricultural Genetic Engineering Research Institute (AGERI), which is part of the Agricultural Research Center (ARC), administered by the Ministry of Agriculture and Land Reclamation. The predecessor of AGERI, the National Agricultural Genetic Engineering Laboratory (NAGEL) was established in 1989.

The research objectives of AGERI are mainly aimed at establishing virus-, insect-, and fungal-resistant crops as well as improving environmental stress tolerance of crops. Another research group aims to increase the nutritional quality of faba bean by isolating and inserting gene encoding for natural storage proteins.

In 1992, AGERI achieved an international breakthrough in genetic engineering of potatoes, by regenerating three commercial potato cultivars with a chimeric gene encoding the coat protein of potato X virus, using the *Agrobacterium tumefaciens* leaf disc transformation method (5c). Other ongoing research activities of AGERI include (72a):

(a) Genetic transformation research on tomato, cucurbits, maize and cotton;
(b) Genome mapping of tomato, rapeseed, and *Bacillus thuringiensis* toxin genes;
(c) Protein engineering of faba bean;
(d) Identification of biotypes of *Bemecia tabaci* (white fly).

Up till 1996, aside from developing transgenic potatoes, AGERI research has resulted in the following (73):

(a) Isolating and sequencing of toxin genes of some Egyptian strains of *Bacillus thuringiensis*;
(b) Field tests of transgenic potato;
(c) Sequencing of tomato virus;
(d) Production of antibodies against gemini-viruses.

Sixteen AGERI senior scientists have institutional affiliations with six Egyptian universities as well as with various national agricultural research centres. Internationally AGERI is collaborating with many universities (including Michigan State University, Cornell University, University of California) in the United States through its ABSP (Agricultural Biotechnology for Sustainable Productivity) project funded by USAID (72b).

Financially, AGERI has been dependent on foreign donors since 1991. UNDP was a major donor in 1991 (US$ 1.5 million (66)), USAID from October 1992 till December 1995 and new USAID funding support has been approved for 1996-1997. The administrating Egyptian institute, the ARC, pays salaries of staff (66,74).

In line with the aim of AGERI to achieve self-reliance and self-financing within AGERI to mobilize the funds necessary for the running costs of laboratories, AGERI has undertaken the following commercial activities (66, 74):

(a) Sale of DNA oligonucleotides (no sale figures);

(b) Sale of ELISA kits to detect potato viruses (in 1995 approximately 50 kits were sold for 1,000 Egyptian pounds (LE) per piece);

(c) Sale of DNA/RNA virus kits for health diagnostic purposes (no sale figures);

(d) Organization of six training courses since 1991, financed by UNESCO - Cairo, FAO-RNEA, NRC, the University of Minnesota and ICARDA.

Other institutes involved with genetic engineering of plants operating with smaller financial and human resources than AGERI include Cairo University and Menoufia University. Table 8 provides a comparison of the most important Egyptian initiatives in genetic engineering.

In May 1995 the Faculty of Agriculture of Cairo University opened a Genetic Engineering Centre. The Centre is still in its infancy as most of the molecular biology equipment is not routinely used for research activities. Instead the research activities for gene transfer are carried out by counterparts in Japan (75). According to the director, the major merit of the new Centre is that it has been established and is maintained with funds from the Faculty of Agriculture. The Faculty of Agriculture provided approximately US$ 2 million for building the premises and provides an estimated US$ 70,000 yearly for salaries and running costs (75,76). Advanced manpower limits itself to three local senior scientists and a Japanese counterpart who is working at the Centre till 1997. Large amounts of equipment for both molecular biology and plant cell and tissue culture techniques were recently purchased. The overall research objectives are similar to AGERI in that the Centre aims to transfer *B. thuringiensis* genes into crops of economic importance in Egypt to install resistance to insects.

TABLE 8. EGYPTIAN PUBLIC RESEARCH INSTITUTES WORKING ON GENETIC ENGINEERING OF PLANTS

Institute	Senior scientists*	Activities started in:	Main objectives	Foreign donor
1. Agricultural Genetic Engineering Research Institute (AGERI). Agricultural Research Center.	16	1989	Research: • Genetic transformation research on potato, tomato, cucurbits, maize and cotton. • Genome mapping of tomato, rapeseed and *Bacillus thuringiensis* toxin genes. • Protein engineering of faba bean • Identification of biotypes of white fly.	(a) UNDP, 1991-1992, $1.5 million (66). (b) USAID, 1993-1995**. (c) USAID, 1996-1998.
2. Genetic Engineering and Tissue Culture Center. Menoufiya University.	4	1990	Research: • Genetic transformation of *B. thuringiensis* genes to cotton.	USAID, 1992-1993, $246,709 (86a)
3. Genetic Engineering Center. Faculty of Agriculture, Cairo University.	4	1995	Research: • Genetic transformation of *B. thuringiensis* genes to Egyptian clover. • Developing micropropagation protocols for faba bean, wheat and Egyptian clover.	JICA, 1994-1997, $1.2 million (75)

* Number of Ph.D. holders.

** Based on an ESCWA visit to AGERI in December 1995, total US contributions for laboratory equipment, bioinformatics (e.g. computers, internet, software), greenhouses and farm equipment were roughly estimated to amount to at least US$ 3 million during the period 1993-1995.

Note: References in parentheses in this table refer to the corresponding references cited in full in the section on "References" in this study.

Cairo University adopted a different strategy from that of AGERI in order to fight the cotton bollworm. Instead of installing *Bt* genes directly into cotton, the co-workers of Cairo University, in collaboration with their Japanese counterparts, are trying to transfer endotoxin genes of *B. thuringiensis* to Egyptian clover, which is a host to bollworm, prior to the cotton growth season.

So far the Centre has been successful in establishing micropropagation protocols for the Egyptian faba bean (77) and wheat. More important, the Centre achieved somatic embryogenesis for Egyptian clover. The mastering of these plant cell and tissue culture techniques is crucial in carrying out the actual gene transfer.

For carrying out genetic engineering research, the Genetic Engineering Centre relies on technical assistance, provided by JICA, which has contributed US$ 300,000 yearly from February 1994 till 1997 as part of a first-term project support to five departments of the Faculty of Agriculture (75). The allotment is spent on general support for the research activities of the Genetic Engineering Centre (including training and equipment). Concerning the overall status of equipment, research achievements and human resources, this centre ranks second in genetic engineering of plants, next to AGERI.

Other less established research institutes working on genetic engineering limit their activities to plant cell and tissue culture activities and testing of equipment. In 1990 the Science and Technology Cooperation (STC) project of ASRT and USAID allocated US$ 246,709 to the Genetic Engineering and Tissue Culture Centre of Menufia University for a genetic engineering project of three years (86a). The Centre was established with technical assistance from the German University of Darmstadt which provided high-level training courses in 1987/88.

Like AGERI and Cairo University, the Genetic Engineering and Tissue Culture Centre of Menufia University is trying to incorporate *Bacillus thuringiensis* endotoxin genes into cotton for pest control. Although the project should have been terminated by end-January 1993, it is still ongoing, owing to delays in obtaining equipment and time-consuming procedures of the STC project administration. Achievements in research are limited to the development of a regeneration protocol of cotton. Research activities in molecular biology are not being carried out. (78,79).

2. *ICARDA*

ICARDA, located near Aleppo, Syrian Arab Republic, is the best established plant breeding institute in the region, specializing in agriculture in dry areas and with worldwide mandates in breeding and germplasm conservation of barley, lentil and forage crops and regional mandates for wheat and chickpea. Biotechnologies used by ICARDA are mainly to support conventional breeding programmes. Biotechnology activities, effectively started in 1992 (104), were initially restricted to the use of genetic markers (RFLP, RAPD, Micro-Satellites) to assess the genetic diversity of the existing *in situ* and *ex situ* collections of mandate crops.

In addition, a search was carried out for genetic markers that can be linked to superior traits such as resistance and tolerance to biotic and abiotic stresses, especially drought. Tissue culture techniques, especially the use of double haploid lines in barley and wheat as well as interspecific hybridization in wheat and chickpea are used to support the breeding programme. The total budget for biotechnology research at ICARDA amounted to US$ 1,335,312 in 1994, of which US$ 617,950 is for salaries and employment costs (101). The total number of staff of the biotechnology laboratory consists of two research leaders and 12 research workers. Funding is mainly supplied by the Governments of France and Germany and by UNDP. ICARDA has subcontracted some of its biotechnology research to German and French universities as well as institutes in Egypt (AGERI), Morocco and Tunisia (104b).

F. Strategies for building expertise in plant genetic engineering

It is clear that genetic engineering is still a very costly enterprise which requires long-term investments, even though the specific results cannot always be predicted, especially in research areas of interest to the ESCWA region, such as crop improvement programmes aimed at installing plant tolerance to drought or salinity. What then are the scientific areas of interest for agricultural biotechnologies in which tangible results can be foreseen? Some options for developing strategies aiming at building expertise in plant genetic engineering are indicated below.

First of all, genetic characterization of plant species native to the ESCWA region is an option for genetic engineering research. This will serve to identify genetically plant species with useful characteristics such as tolerance to aridity, salinity and low soil fertility; such tolerance is invaluable in this part of the world. Research groups in more developed parts of the world are not in a position to patent genes isolated from such plants. In addition, the endangered species identified can be more effectively protected, using tissue culture- and gene-banks. An added advantage is that the use of genetic markers in crop breeding programmes might reduce the 10-15 gene breeding cycles by 2-3 years.

In other words, genetic characterization of native plant species will provide means a for protecting biodiversity and supporting traditional crop breeding programmes as well as developing scientific know-how in genetic engineering. Genetic mapping techniques to characterize plant genomes are becoming standard procedure and therefore form a good basis to start a research programme in genetic engineering.

In addition, molecular biology techniques offer an added advantage for biodiversity conservation. Instead of protecting biodiversity through direct control (i.e. *in situ*), it may be more practical to offer services that combine genetic resources with capacity in science and technology.

A second area of interest, with more direct commercial interest, would then be the development of plant gene transfer technology for single gene traits. This is quite possible with currently available technology, provided an experienced research group is established. An example is installing *Bacillus thuringienis* genes into plants, whereby the plant will produce its own pesticides against a broad spectrum of insect pests. Finally, and strategically important for the long term, complex genetic traits such as tolerance to aridity can be characterized. A basic understanding of the molecular genetics of such characteristics will provide insight into possibilities for manipulating the related genetic system of tolerance against aridity.

It must be emphasized that any kind of technological development of genetic engineering should address all areas to some extent. Research in molecular biology will always depend on living entities and is therefore always a question of trial-and-error to a certain degree. Therefore one can best focus on a crop native to the region, of economic importance, and address both molecular genetic processes affiliated with tolerance to abiotic stress, resistance to pests and diseases and characterization studies for biodiversity identification purposes. Other research areas, such as increase in yield, better shelf-life or taste of produce as well as revolutionary applications such as genetic manipulation of crop species to produce new products (e.g. enzymes and other biochemicals and even plastics (34)) should also be considered.

Should impressive results be achieved in one area of research, there should be flexibility in the entire programme to allow for changing the emphasis of the entire research programme to that area, without, of course, neglecting the others.

So far, options for genetic engineering of plants have been described from a research point of view. However there are other possibilities for technology transfer of genetic engineering. At the other side of the scale of possibilities is direct purchase of genetically engineered plant germ plasm. This might offer a solution in the short term to some problems in the region, as the first genetically engineered crops have been cultivated in the United States and are about to be sold on the international market; they are only restricted by pending United States Government regulations, at present.

It is questionable, however, whether the companies marketing the plant germ plasm are interested in selling their produce in developing countries. As patent law, with regard to genetically transformed plant species, is still controversial in the United States itself, it will require a lot of effort to establish marketing channels of genetically transformed crops in the region. In addition, most developing countries do not have clear patent and licensing laws, and illegal duplication and distribution is the rule rather than the exception. Of course, as international marketing of genetically engineered crop produce and seeds is still unprecedented, this option of technology transfer has to be thoroughly investigated.

Between the opposing ends of technology transfer through direct purchase and in-house research is a whole range of possibilities comprising part of both options. If an alternative is chosen combining the development of regional research capacities with obtaining established research protocols or products, the focus of any such strategy should be to ensure a solid funding position. A long-term substantive funding position is crucial for embarking on genetic engineering research, as large investments are required and results of research are still unpredictable.

For instance, the United States biotechnology industry is still operating at a loss, despite the fact that the United States Government finances private and public biotechnology research for more than US$ 4 billion annually (i.e. half of the total budget spent on biotechnology research) and has been doing so for the past

20 years. For the 15 main agrobiotechnology companies operating in the United States, the average of R&D in relation to revenues is 106%. The reason for this high percentage is the low or absent revenues for most of the companies in combination with the large and still growing investment in R&D. McKell describes a relationship between the complexity of the biotechnology and the time required to develop products. The most complex technologies, such as production of genetically transformed plants by DNA transfer, require considerable time and scientific effort. In most cases, developing countries will find that they will get the largest return from their investments in the more traditional—and proven—biotechnologies (14b). Regarding genetic engineering, ESCWA member countries can best exploit and investigate possibilities for crop improvement through regional or international public research centres.

At present, Egypt, Iraq, Kuwait and the Syrian Arab Republic are affiliated with the International Center for Genetic Engineering and Biotechnology (ICGEB), an autonomous international research institute, supported by its member States. Two research institutes of ICGEB are operational in Italy and India. An institute such as ICGEB could well function as a vehicle for establishing genetic engineering research on relevant issues for the ESCWA region. In addition to carrying out advanced biotechnology research, ICGEB aims to act as a catalyst for technology transfer (35a).

An alternative might be collaboration of the region with crop breeding programmes undertaken with any of the CGIAR (Consultative Group on International Agricultural Research) research centres. These research centres are addressing all three stages of crop breeding, including genetic engineering options for crop improvement. Of those, ICARDA might be the most appropriate. Yet another alternative might be the Agricultural Genetic Engineering Research Institute (AGERI) of the Agricultural Research Center in Egypt, partly established through UNDP and USAID funding. At present, AGERI is the best established institute in the region for research, development, networking facilities and training related to genetic engineering of plants for crop improvement.

Concerning the human and financial requirements for genetic engineering, Western Asia has a considerable advantage over other developing regions which have not established solid genetic engineering R&D programmes. Some countries in the ESCWA region have highly qualified human resources. Other countries, the Gulf countries in particular, have large financial resources to meet the challenges of genetic engineering in the long run. In the past decade, the Gulf countries have shown continued interest in increasing agricultural production (27,28,29), and have allocated generous government subsidies at both the input and the output level (18). Therefore, to consolidate these resources, regional collaboration could well be the key to establishing genetic engineering technology in the region.

II. PLANT CELL AND TISSUE CULTURE

The technology of plant cell and tissue culture (PCTC) evolved from the capacity of a single plant cell to redifferentiate into a new plant. This capacity, or so-called totipotency is unique to the kingdom of the Plants and was first described by Haberlandt in 1902. Tissue culture techniques make use of parts of a plant (e.g. leaf, stem, root, meristem) to regenerate a new plant, whereas cell culture techniques make use of a liquid medium containing single plant cells. Cell culture can be used for producing secondary metabolites or artificial seed.

The first plant to become fully dependent on tissue culture for its propagation is the orchid. Under natural circumstances orchids can only germinate by means of a symbiosis with a specific fungus generally referred to as mycorrhiza. In 1922, Knudson demonstrated that orchid seed germination was possible on a simple nutrient medium containing minerals and sugars without the help of any fungus (97a).

Soon thereafter this discovery was put into commercial use by the Singapore Botanic Gardens in 1928. Today orchids are grown by the assembly-line method in extensive glasshouses with controlled environment, and the sale of orchid flasks runs into millions of US dollars. Orchids account for 2.7% of global cut flower production in terms of value. Orchid flower sales in the United States, for domestic consumption alone, are valued at US$ 50-60 million. Thailand alone exported orchids worth more than US$ 26 million in 1993. More than 700 orchid growers and about 20 commercial tissue culture laboratories in Thailand are the basis for this success. In addition to enabling germination, orchid tissue culture has also resulted in the recovery of disease-free clones, the preservation of valuable germ plasm and the conservation of many species that were on the verge of extinction (97b).

In the broadest sense, tissue culture is merely an extension of vegetative propagation routinely carried out in a commercial nursery by the rooting of stem cuttings or other plant parts or induction of shooting from root cuttings in a sand culture. Tissue culture has the major advantage of potentially providing hundreds or thousands of identical copies of plants in a small space.

The practical implication of this capacity is that tissues and cells from one superior plant can be used to produce thousands of plant propagules with the same genetic composition. Further, micropropagation of a plant can take place without the delay required for it to bear fruit and seeds. Cell and tissue culture techniques can also be used to induce mutations by the use of selective media or by fusion of plant cells, thereby contributing to crop improvement schemes.

As with genetic engineering, one important application of plant cell and tissue culture is for crop improvement. PCTC takes place at the cellular level of crop improvement, which implies that the potential for crop improvement is not as spectacular as that being envisaged for genetic engineering, as barriers between different species can be fully traversed by genetic engineering.

This does not, however, imply that PCTC is expected to disappear very rapidly as the core technology for fast improvement of plant species. This is mainly due to the fact that PCTC constitutes a general prerequisite for attempting genetic transformation of plants. The creation of genetically engineered plants requires equal contributions from two technologies: *in vitro* culture (i.e., PCTC) and gene transfer. Only in cases where optimization of both technologies has taken place, in a concerted fashion, has creation of transgenic plants become practical. Even in cases when a report describing engineering of a specific species appears in the literature, it does not necessarily mean that the procedure is applicable to a wider germ plasm including elite cultivars nor that it is practical and straightforward (37). The technology of gene transfer of many plant species has recently leaped forward, leaving the recovery of plants from transgenic cells as a major limitation (38a). In other words, PCTC technology, like genetic engineering, still harbours many enigmas.

Another resemblance between PCTC and genetic engineering is that both technologies have limitations as to their potential contribution to agriculture. The widespread and large-scale use of genetically homologous plants may well result in a higher vulnerability of the overall crop to pests, diseases and abiotic stress. In turn, this would again increase the use of pesticides and other chemicals.

Tissue cultured plantlets are also considerably more expensive than traditionally grown plants. In 1983 Unilever's cloned oil-palm plantlets were 18 times more expensive than the normal ones (199f). A more serious risk of using tissue culture for *in vitro* germ-plasm conservation is related to the occurrence of spontaneous mutation during regeneration. This so-called "somaclonal variation" may cause preserved material to undergo genetic changes whereby the original plant variety is lost.

A. Research areas

Foremost, plant cell and tissue culture is a technology which facilitates the rapid multiplication of superior plant material. Other application areas are related to plant-breeding programmes, the production of disease-free plants, germ-plasm conservation, the regeneration of transgenic plants (see chapter I), and the production of secondary metabolites. In contrast to plant genetic engineering, plant cell and tissue culture techniques have more diverse applications. Table 9 presents an overview of PCTC research areas and techniques.

1. *Micropropagation*

PCTC has become an indispensable tool for rapid regeneration and the multiplication of superior genotypes (micropropagation), without the risk of losing important genetic traits. Micropropagation protocols are currently available for most species of commercial interest. Micropropagation is specifically advantageous for those species that produce little or no viable seeds (e.g. ornamentals, perennials, tuberous plants). Woody perennials such as grape, fig, date palm, almond and pistachios are of particular interest to the ESCWA region. Woody species are, however, far more difficult to clone than herbaceous species. Vegetative propagation of many monocotyledonous palms and forest species is virtually impossible (36a).

In contrast, tropical fruit herbaceous species such as banana and pineapple can be commercially propagated *in vitro*. For citrus trees, the most promising option is the micrografting of meristems onto seedling rootstocks *in vitro*, to obtain *Citrus tristeza* virus-free planting material. In general, the use of micropropagation (or vegetative propagation through tissue culture) is a major alternative to conventional methods of *in vivo* vegetative propagation, which is comparatively more expensive, slower and more complicated.

The major stages for carrying out micropropagation are the following:

(a) Selection and maintenance of stock plants for culture initiation;
(b) Establishment of aseptic culture;
(c) Multiplication of shoots or somatic embryogenesis;
(d) Rooting of regenerated shoots *in vitro* or germination of somatic embryos;
(e) Transfer of plantlets to sterilized soil for hardening under greenhouse environment.

The adoption of all of these techniques or stages not only simplifies the daily operation, accounting and production costs, but also allows for greater ease in communication with other laboratories. Thus a particular plant can be marketed or requested by specifying its stage.

TABLE 9. PCTC RESEARCH AREAS AND TECHNIQUES

Research Area	Purpose	Techniques	Applications
Micropropagation	*In vitro* vegetative plant propagation for plants which do produce limited viable seeds	• Proliferation of meristems • Callus culture • Organogenesis/ somatic embryogenesis • Rooting/ germination	• 1960, G. Morel: Only commercial approach for orchid propagation • For woody perennials (e.g grape, fig, date palm, almond, pistachios, timbers, rubber, coffee, tea)
Breeding Programmes	The induction of optimum genetic variability of germ-plasm sources	(i) Haploid production (ii) Triploid production (iii) *In vitro* pollination (iv) Ovule and embryo rescue (v) Somatic hybridization (protoplast fusion) and cybridization (genome-plastome fusion) (vi) Somaclonal and gametoclonal variant selection	(i) Reduces time-frame of breeding programmes (ii) Triploids are seedless, which increases edibility of fruits (iii) For hybrid embryo production (iv) For interspecific and intergeneric hybridization[a/] (v) For producing hybrid plants of parents that cannot cross by conventional methods of plant breeding (vi) Increase genetic variability using PCTC
Eradication of disease factors	Mass production of disease-free plants	(i) Meristem-tip culture (ii) Heat treatment or chemical treatment (iii) Callus culture (iv) Micrografting	(i) Isolate plant part which contains little or no virus (ii) Inactivates virus (iii) Isolate rapid duplicating cells which contain little or no virus (iv) To overcome rooting problems; graft meristem on a virus-free root stock (especially with perennials such as citrus)
Germ-plasm Conservation	To preserve the maximum possible genetic diversity of vegetatively propagated plants	(i) Freeze-preservation (ii) Cold storage (iii) Low-pressure and low-oxygen storage	(i) Preservation of tissue or cell cultures (ii) No cryogenic injuries as with i (iii) Alternative to i and ii
Production of Seconda y Metabolites	For industrial level production of flavours, fragrances, essential oils, pigments, sweeteners, feedstocks, antimicrobials and pharmaceutical	(i) Free cell suspension culture (ii) Immobilized plant cell culture (iii) Two-phase system culture (iv) Biotransformation	(i) Mass cultivation of single plant cells producing high concentrations of secondary metabolites (ii) Optimization of i (iii) Facilitates removal of the product (iv) Conversion of a small part of a chemical molecule

a/ M. Baum, Research Team Leader Biotechnology, Aleppo, Syrian Arab Republic, Personal Communication.

The actual plant cell and tissue culture techniques are largely confined to stages (iii) and (iv). The multiplication of shoots and subsequent regeneration of shoots is termed "organogenesis". The formation of organs is achieved by culture media containing plant hormones in specific concentrations.

The principles of axillary shoot formation were already known in 1925. The alternative to organogenesis for micropropagation purposes is somatic embryogenesis, which was first developed in 1958 (38b). Somatic embryogenesis is the process whereby somatic cells develop through the stages of embryogeny to give whole plants without gamete fusion. It is the ability of repetitive embryogenesis to perpetuate the embryogenic state indefinitely and produce large numbers of embryos that makes somatic embryogenesis a powerful tool capable of being exploited for diverse goals such as mass propagation and

the production of transgenic plants (38). With many plant species, however, induction of embryogenesis is often very difficult, or has been impossible up till now. Genetic variability of clones is usually larger with somatic embryogenesis. Shoot organogenesis is therefore used almost exclusively for commercial micropropagation.

2. *Plant breeding programmes*

Plant breeders are, most of all, interested in obtaining a maximum genetic variability of germ-plasm sources. Typical plant species originating in the ESCWA region are characterized by a small genetic diversity, such as with the graminaceous (e.g. wheat, barley) and leguminous (e.g. chickpea) families. There is a wide range of PCTC techniques which can assist the plant breeder in optimizing the use of his germ plasm resources (see table 9).

Tissue culture can be used for creating homozygous diploid cell lines from haploid plant cells, isolated from microspores in the anther. This technique is generally referred to as the production of double haploids. This is especially beneficial in the case of self-pollinating crops as is the case with *Gramineae* (e.g. barley, wheat). There are reports on haploids being induced by anther-microspore culture from about 250 species and hybrids (including rice, maize, brassica, pepper and tobacco) (36b).

The basic advantage of double haploids is that gene actions can be directly linked to a single allelic dose present in chromosomes of an entire genome. The doubled haploid system, which reveals potential variations of polygenic traits (such as yield and abiotic stress tolerance) within one cycle of recombination by combining anther culture and sexual hybridization, is especially promising for raising superior homozygous breeding material. One limitation of haploid production is that anther culture has not proved successful for all genotypes of crop species.

PCTC can also be used for the production of triploid plant breeding material by endosperm culture. After fertilization in diploid plants, the endosperm is usually triploid. By culturing this endosperm *in vitro*, one can regenerate triploid plants. Triploids are self-sterile and usually seedless. This trait increases edibility of fruits and is desirable in such species as citrus, apple, banana, mulberry, grape, mango and watermelon. Triploid production through endosperm culture has been successful only in a limited number of species, of which citrus holds the most noteworthy commercial application.

Ovule- and embryo-rescue techniques offer an opportunity for producing hybrid embryos among plants that cannot cross by conventional methods of plant breeding. Applications of *in vitro* pollination are mainly in overcoming self-incompatibility (as with *Petunia spp.*) and cross-incompatibility. The latter enables interspecific, intergeneric and interfamilial crosses. Such hybrids have been obtained with a limited number of species, such as brassica, tobacco, wheat and maize. The technique still poses a lot of practical difficulties. More important, although interfamilial crosses have been demonstrated, the seeds are not viable. For commercial breeders this is important as it limits unauthorized multiplication of seeds.

Zygotic embryo culture, or embryo rescue, is the culturing of sexually produced embryos *in vitro*, with the objective of obtaining viable plants. Embryo rescue can be applied to obtain interspecific and intergeneric hybrids, which abort owing to endosperm barriers. This has been achieved in many species (e.g. brassica, chrysanthemum, barley, rice, many legumes) (36). It is especially a worthwhile technique in attempting wide crosses with wild relatives to increase the genetic variability of germ plasm (as with barley and wheat). Other applications of embryo rescue are in overcoming dormancy, shortening of the breeding cycle (in iris and *Rosa spp.*), production of haploids (in barley) and in micropropagation (conifers). Noteworthy is the cultivation of "Makapuno" coconuts using embryo culture, which are very expensive

because of their characteristic soft fatty endosperm in place of a liquid endosperm. In general, embryo culture is, however, still difficult and much research is needed concerning media requirements.

Somatic hybridization or protoplast fusion is mainly applied to overcome cross-incompatibility, as with *in vitro* pollination. The initial prospects of protoplast fusion seemed very promising for increasing genetic diversity (36c). In retrospect, however, the view that protoplast fusion would be able to bypass genetic barriers was very naive. If genomes are incompatible, they are incompatible no matter how they are brought together. In cases in which fertile hybrids were obtained through protoplast fusion, they could also have been obtained sexually or through embryo rescue (83a).

Another breeding application which showed great promise initially is somaclonal variation. Individual plants, grown *in vitro*, can become genetically variable from their original genotype resulting in superior varieties. These superior varieties can then be mass-propagated using PCTC. Somaclonal variation still poses the problem of instability of the superior traits when plants are grown to full maturity. Overall, somaclonal variation is not regarded as the most efficient way to go after variability: it is expensive and the results are random (83b). There are, however, some cultivars (tomato, pepper) on the market which were obtained through somaclonal variation.

3. *Production of disease-free plants*

A third area in which PCTC currently offers a major solution is the multiplication of disease-free plant material by vegetative propagation. Many root and tuber crops are normally propagated from divisions of the tubers, and thus any residual effects are also propagated when the plant material is subdivided. Through heat treatment of the meristematic tissue in shoot tips by a process called thermotherapy, virus-free tissues can be obtained which in turn can be mass-propagated. An alternative is chemotherapy whereby the virus is eradicated by the use of anti-metabolites. This technique is now effectively used for virus eradication in many crops (e.g. potato, ornamentals, cauliflower, soybean, asparagus, tobacco).

The production of virus-free potato seedlings as a commercial enterprise is the most widely used application of producing disease-free plants, both in industrialized countries as well as in developing countries. The initial costs for using tissue culture in potato multiplication are easily recovered in the value of the fifth generation tubers (80a).

4. *Germ-plasm conservation*

Another conventional application area of PCTC is *ex situ* germ-plasm conservation in order to preserve the maximum possible genetic diversity of a particular plant or genetic stock for future use. *In vitro* conservation techniques are particularly of interest for those plant species that are difficult to keep as seeds, such as many fruit tree species, or vegetatively propagated crops such as potato. Advantages of *in vitro* conservation include the following (36d):

(a) Requirement for little space for preservation of a large number of clonally multiplied plants;

(b) Assurance of a pest- and pathogen-free environment;

(c) No influence of natural environmental hazards;

(d) Availability of nucleus stock to propagate a large number of plants rapidly, whenever necessary.

A major drawback of *in vitro* conservation is related to the need of subculturing, whereby loss of germ plasm or invasion of a pathogen may occur. Another cause for loss of plant genetic material may stem from genetic instability of the preserved material. This is particularly observed in plant material which is in an undifferentiated phase (cell or callus culture).

The maintenance of the material as plantlets and the subsequent propagation from their nodal cuttings reduce the risk of genetic instability. An alternative is the storage of somatic embryos, which carries the risk of a high degree of genetic stability. A basic requirement for practical feasibility of a plant tissue culture method in germ-plasm conservation is to reduce the frequency of subcultures to the bare minimum. This can be achieved by freeze-preservation, cold storage, low-pressure and low-oxygen storage.

Freeze preservation is the most reliable for the long-term preservation of cell cultures which are used for the synthesis and accumulation of secondary metabolites. Cold storage appears to be the most cost-effective and easy way for preserving germ plasm *in vitro*. This has been particularly successful in fruit-tree species. Virus-free strawberry plants can be maintained for at least six years at 4°C, and over 800 cultivars of grape plants were reported to have been stored for over 15 years (36e). A wide range of species can now be stored for 6-24 months by maintaining their shoot cultures in slow growth at cold storage.

5. *Regeneration of transgenic plants*

More advanced research areas of PCTC are confined to regeneration of whole plants from cells transformed by recombinant DNA techniques (see chapter I). Somatic embryogenesis is currently the most promising PCTC technique for supporting genetic engineering in that once plant material has been transformed, it can be propagated continuously to produce large numbers of embryos. Plants that have been transformed via indirect somatic embryogenesis include alfalfa, cotton, carrot, eggplant and sunflower.

B. Financial arrangements and developments

In contrast with genetic engineering, plant tissue culture technology appears to be more within the reach of developing countries. Why is tissue culture more accessible for developing countries? The most important reason is the combination of fewer requirements for investment and research together with much better prospects for a direct return on investments. The latter is seen as a crucial factor, especially for the private sector in many developing countries. Time requirements to set up a tissue culture production unit are around 6 - 12 months (105a).

The additional economical advantages of micropropagation in comparison to *in vivo* vegetative propagation are so numerous that it appears to be the most obvious biotechnology to suit the potential and needs of the least developed countries. Additional benefits of micropropagation, aside from a relatively short gestation period, include (118a):

(a) Growth of *in vitro* propagated plants is often stronger than in those cloned *in vivo*; this is mainly due to rejuvenation and/or the fact that plantlets are disease-free;

(b) Micropropagation can replace expensive methods such as grafting or budding on a rootstock;

(c) In contrast to *in vivo* micropropagation, *in vitro* cloning of herbaceous plants can be continued all year round and so become independent of the seasons;

(d) Micropropagation enables the production of disease-free plants and thereby facilitates phytosanitary transport from country to country, allowing for good export opportunities;

(e) Costs of greenhouses are less with micropropagation as few stock plants are required as starting material and much less greenhouse space is required for making cuttings.

As a result, private companies working on tissue culture have sprung up in many developing countries. And indeed, any country where a crop species is, at the same time, a major staple food could profitably invest in a tissue culture laboratory. At a capital cost of about $20,000 (in 1993), this would permit the import, as tissue cultures, of virus- or disease-free clones developed in research stations abroad, and their rapid multiplication, if they proved adaptable to local conditions and acceptable to producers and consumers (5d).

Undoubtedly, micropropagation has many advantages, but in most cases it is still much cheaper to multiply many plant types from seeds or to multiply them vegetatively (172a). In the experience of commercial horticultural nursery companies in the United States, large-scale propagation *in vitro* is justified economically only in cases in which:

(a) Propagation by cuttings is costly and difficult (as with perennials);

(b) Propagation by seeds would involve undesirable genetic diversity;

(c) The end-product has a high unit value (date palm, potato, banana);

(d) It is necessary to separate known genotypes in plants that combine male and female in one plant, from plants that separate the sexes in different plants (e.g. oil palm, date palm, jojoba, pistachio).

It must be emphasized, however, that the market for tissue-cultured plants is far from exhausted. So far, the demand for micropropagated plants has always been higher than the production. For instance, the Netherlands produced 50 million plants in 1986 against a demand of 85 million plants. In 1991, the demand had gone up to 250 million against a production of 80 million plants. Total sales of plants micropropagated in Europe amounted to US$ 67 million in 1990. Extrapolating this figure to worldwide production figures, the total turnover of micropropagation businesses can be estimated to be approximately US$ 200 million (172b). By the year 2000, the global potential demand for tissue-cultured plants is roughly estimated to be worth about US$ 15 billion (124).

As can be seen in table 10, commercial micropropagation is well-established on a worldwide scale. However, most initiatives are for the multiplication of ornamental plants and the production of cut flowers. Most firms in the United States and Western Europe were established in the 1970s and 1980s. Laboratories propagating orchids then became established in Asia, and now the micropropagation of a wide range of plants has been started in Eastern Europe and Asia. Meanwhile the number of businesses operating in the United States and Western Europe declined. In 1993 it was estimated that only 170 commercial laboratories were active in Western Europe, compared with 248 in 1988 (172c).

Micropropagation for the purpose of providing plantlets of similar size on a short notice is another major area where commercialization has taken place. Especially vegetatively propagated plants (e.g. potato, banana, strawberry, pineapple, fruit trees and palms) are promising as *in vitro* propagation will always outcompete *in vivo* propagation in time requirements. For clonally propagated crops (such as banana, cardamom, pineapple, potato) micropropagation can virtually double the yield. Other commercial opportunities are for shortening time requirements for plant breeding by several years from the usual 6-10 years time frame required for establishing a new crop variety.

TABLE 10. COMMERCIAL GLOBAL MICROPROPAGATION BY REGION IN 1988

Region	No. Commercial laboratories	Millions of plants produced	Principal crops
Africa	n.a.	n.a.	n.a.
Asia	105	92	Orchids, cut flowers
Australia and New Zealand	20-25	82	Ornamentals, foliage plants and cut flowers
Eastern Europe	162*	34	Foliage plants, cut flowers
Middle East	n.a.	6**	n.a.
Middle and South America	15-20**	n.a.	Mainly ornamentals
North America	100	115	Foliage pot plants, woody plants
Western Europe	248	212.5	Pot plants, cut flowers
TOTAL	650-660	541.5	

Sources: E.F. George, "Commercial micropropagation", *Plant Propagation by Tissue Culture: In Practice* (Exegetics Ltd., 1996, United Kingdom), part 2, p. 793; and R.L.M. Pierik, "Micropropagation: technology and opportunities", *Plant Biotechnology, Commercial Prospects and Problems* (Andover, Hampshire, United Kingdom Intercept Ltd., 1993), p. 11.

* Information imprecise. Many small laboratories were previously government-supported.
** Low estimate.

Flowers and ornamental plants have been targeted most for commercial micropropagation. This is mainly because the market of flowers and ornamental plants does not demand a certain degree of heterozygosity of produce, in contrast to markets for agricultural crops and forestry. In 1988 ornamentals accounted for 74% of tissue cultured produced plantlets in Western Europe (118c).

However, flower cuttings of many common plants mass-produced by conventional methods are still much cheaper than micropropagated material. For example, the price for chrysanthemum tissue culture plantlets in the United Kingdom amounts to US$ 0.24, which compares unfavourably with the price of cuttings produced in the Canary Islands (i.e. US$ 0.08) and imported into mainland Europe (172d). Table 11 indicates the ranking of the most promising flowering and ornamental plants for commercial micropropagation in terms of turnover.

It is difficult to estimate the economic viability of commercial *in vitro* propagation. Much is dependent on the plant species of interest and the availability of a suitable tissue culture protocol. Although nowadays protocols are available for most plant species, not all methods are equally suitable for commercial micropropagation. Most farmers prefer superior varieties and thus a low level of genetic variation from the original plant material is desirable. Such variation is most likely to occur with those tissue culture techniques which give the highest production number of progeny, such as somatic embryogenesis, callus and cell culture systems.

TABLE 11. TOP 10 FLOWERING AND ORNAMENTAL PLANTS IN THE TRADE OF MICROPROPAGATION IN THE DESCENDING ORDER OF THEIR TURNOVER

Flowering Plants	Ornamental plants
1. Rose	1. *Ficus*
2. *Chrysanthemum*	2. *Dracaena*
3. Carnation	3. *Begonia*
4. Tulip	4. *Saintpaulia*
5. *Freesia*	5. *Yucca*
6. *Gerbera*	6. *Azalea*
7. Lily	7. *Poinsettia*
8. Orchid	8. *Kalanchoe*
9. *Gypsophila*	9. *Dieffenbachia*
10. *Iris*	10. *Cyclamen*

Source: M. Rathnavel and J. Prakash, "Commercial production: criteria, economic and bottlenecks, *Plant Biotechnology, Commercial Prospects and Problems* (Andover, Hampshire, United Kingdom Intercept Ltd., 1993), p. 25.

If investment capital permits, it is obvious that large-scale production will be targeted. Investments made in most of the capital equipment do not vary according to the volume of production. Large-scale production would allow for a wider range of plant species to be propagated. This assures flexibility in adopting to market demand. Generally, competition is severe between micropropagation companies, as it is a production-based industry with the ability to produce large numbers of plants well beyond demand. Demand for plantlets is limited to specific time intervals during the year and is related to the cropping season of such crops. By focussing on a broad range of plants with different peak seasons, facilities and manpower can be better utilized throughout the year.

Large-scale operations have an additional advantage in that the potential market can be increased by reducing the average production costs. A 1991 estimate suggested that a 50% reduction in average cost would allow the market to be expanded to more than 10 times its current size. By decreasing production costs by 90%, the potential market would become 1,000 times larger than at present (172e).

Major commercial viable opportunities for agricultural crops are to be found with a limited number of plant species such as potato, banana, strawberries, and fruit trees. Potato, banana and fruit trees are generally vegetatively propagated and viral diseases are therefore easily transmitted to planting material, resulting in crop produce losses of up to 40%. Micropropagation of these species has received most attention since viruses can only be eradicated effectively with the aid of tissue culture. World market prices of elite virus-free potatoes amounted to US$ 0.60 per mini-tuber in 1995 (i.e. approx. US$ 6-12 per kg) (125).

Initial costs for outfitting a tissue culture laboratory for mass-scale virus-free potato tuber production is estimated to amount at least to US$ 185,000, excluding supplies (see table 12). Major initial costs for virus-free potato production are related to the need for an advanced greenhouse, amounting to approximately US$ 600,000 for 1,000 m^2 in 1995 (87). Such greenhouses are necessary to avoid virus contamination (transmitted through aphids) of the shoot-tip propagated planting material used to obtain the virus-free minitubers. Once established, most of the running costs for a laboratory consist of labour costs and therefore vary from country to country.

TABLE 12. PRICES OF KEY EQUIPMENT OF A PCTC SUPPORTIVE LABORATORY FOR MASS-SCALE PRODUCTION OF VIRUS-FREE POTATOES

Equipment	Costs in US$ (1992)
4 biosafety hoods	28,000
4 growth rooms (totalling 50 m^2)	44,000
1 autoclave	44,000
ELISA reader	9,000
ELISA washer	11,000
Analytical and regular balances	7,000
1 freezer	1,000
1 fridge	1,000
Microscope	15,000
2 incubators	10,000
TOTAL	185,000

Source: Z. Csizer, "Design, Construction, Development and Management of NIGEB. Comments and Recommendations on a Pre-proposal and Results of Discussions with the Study Team of the Academy's Focal Point", UNIDO consultant's report, Cairo, 1994, pp. 10-11.

In the case of the Syrian Arab Republic, the General Organization for Seed Multiplication (GOSM) started mass-scale production of potatoes in 1995 and spends US$ 80,000 for running costs (including labour) of the laboratory; ultimately 200 tones of virus-free potatoes were produced (87). Based on a cost model of Chu and Kurtz (see table 13), indicating relative cost components associated with micropropagation, the total running costs of this Syrian PCTC production line would then amount to approximately US$ 500,000 per year. For other cost models the reader is referred to Stanart de Metsenaere (124c), who developed a formula for calculating the cost price and the minimum selling price of producing micropropagated plantlets, taking into account multiplication factors and losses due to contamination. George (172), provided a more detailed analysis in 1996.

TABLE 13. RELATIVE COST COMPONENTS ASSOCIATED WITH MICROPROPAGATION OF A TYPICAL CROP

Cost component	Percentage
Laboratory (direct labour)	15
Utilities	9
Depreciation	7
Supervision (laboratory and greenhouse)	23
Planting (direct labour)	9
Other production costs	37
TOTAL	100

Source: M.K. Razdan, An Introduction to Plant Tissue Culture (New Delhi, Oxford & IBH Publishing Co. Ltd., 1993), p. 285.

On the Syrian market the average price of potato planting tubers amounts to approximately US$ 0.30 per kg, resulting in net sales of US$ 60,000 over 1995. By the end of 1996 GOSM aims to produce 7,000 tons of potato tubers using PCTC, by which a return on investments could be obtained (87). If GOSM had access to the world market, the 200 tons of elite virus-free potatoes would yield US$ 1.2 million to 2.4 million, ensuring net profits of US$ 0.7 million to 1.9 million in the first year of production alone. Of course, such spectacular profits are not to be expected in such a short time-span.

The production of virus-free potato mini-tubers in developing countries would also result in considerable savings in foreign exchange. It was estimated that Egypt could save up to US$ 30 million per annum through local production (72c). Imports of potato seed tuber in Jordan amounted to approximately US$ 3.4 million in 1993 (126a).

It is not an exception for small private PCTC companies in non-industrialized countries to reach the financial break-even point in a couple of years. For example, Fitotek Unggul, a small propagation company established in Indonesia in 1987, managed to obtain a return on investments within four years. Currently Fitotek operates with approximately 115 staff, 175 square metres of growth room space and 20 laminar flow stations (89).

The company supplied 7% of Indonesia's demand for banana plantlets in 1993. Other propagated crops include tobacco, vanilla, pineapple and ornamentals. About 90% of the company's contracts are local (of which 70% for pineapple, banana and vanilla and 30% for ornamentals), whereas 10% of contracts are mostly with New Zealand and Netherlands firms.

Viet Nam benefited from technological expertise from Taiwan Province of China in establishing the commercial micropropagation of banana plantlets (135). The joint venture between a Taiwanese company (Pan Viet Company) and the Biotechnology Research Center of Viet Nam produced 4 million micropropagated banana plantlets in 1991.

Nepal, one of the poorest countries in the world in terms of GDP, has developed a modest tissue culture industry. Three private companies have been mass-producing ornamental plants and strawberries on a small scale since 1992. Large-scale production of virus-free potato mini-tubers by the Ministry of Agriculture has been realized with technical assistance from the Swiss Development Cooperation. In 1994 this production recorded a market share of approximately 10% of the total demand for potato seed tubers in Nepal (125).

Of all crops grown in Western Asia, date palm may well benefit most from tissue culture to increase production of dates. Plants can be raised from seed, but are then variable and half their number become unproductive males. The male and female plants cannot be distinguished until they have been grown in the field for several years. Dates are only produced by female plants.

Date palm plantations are usually established through vegetative propagations and normally consist of one or more clones of mainly female trees. Traditional vegetative propagation is slow, with only 20 offshoots produced per plant during its first five years in the field. On planting the offshoots, only 50% will grow roots and develop into a full plant. This rate has proved inadequate to extend date palm cultivation and to replace aging plantations, or those destroyed by Bayoud disease caused by *Fusarium exosporium* f. sp. *albedinis* or the red weevil. The red weevil (*R. ferrugineus*) causes particularly severe damage to date palm estates in Saudi Arabia. Two methods are available for *in vitro* multiplication of date palm: shoot culture and somatic embryogenesis. For commercial micropropagation, shoot culture is the preferred method (173a) as the risk of genetic variation is considered less than with somatic embryogenesis.

The first report of clonal multiplication of date palm was made in 1979 by Poulain (173b). Over the last decade micropropagation of date palm has played a key role in making more plants available to growers in the region. In most cases micropropagated plantlets have been imported from outside the region.

Twyford Plant Laboratories Ltd., a British-based company, managed to propagate 10,000 plantlets of date palm which were sold at a unit price of US$ 20 to Saudi Arabia, Oman and the Islamic Republic of Iran, in 1986. The plantlets were obtained through somatic embryogenesis (7a). DNA markers have been developed to check that the genome of the plantlets is identical to that of the mother plant.

In 1996, the Institute National de la Recherche Agronomique (INRA) of Morocco reported the successful development of a shoot tissue culture protocol for date palm. The INRA laboratory produces the starting cultures, and a private company ensures large-scale micropropagation and acclimatization of plantlets. In recent years 170,000 micropropagated date palm plantlets have been distributed to farmers in Morocco (131). According to Sasson (7b) INRA benefited considerably from collaboration with the French oil company Total Compagnie Française des Pétroles by undertaking a joint venture for developing tissue culture protocols for date palm.

Date palm (*Phoenix dactilifera*) is the most important crop for Western Asia. The region has been responsible for 57% of total world production for many years (see table 14). The more traditional producers of dates include Iraq and Egypt, with more than half a million of metric tons of dates produced annually. Over the last decade Saudi Arabia witnessed the fastest growth in production of dates and has joined this list of major producers.

TABLE 14. PRODUCTION OF DATES IN ESCWA MEMBER COUNTRIES

Country/area	Date production 1,000 MT			
	1979-81	1992	1993	1994
Bahrain	31	18F	19F	19F
Egypt	414	604	631	600F
Iraq	495	590F	613	600F
Jordan	-	-	-	-
Kuwait	1	1F	1F	1F
Lebanon	-	-	-	-
Oman	70	130F	133F	139F
Palestinian territories	2	3*	2F	2F
Qatar	4	10	11	11F
Saudi Arabia	377	550	563	564F
Syrian Arab Republic	-	-	-	-
United Arab Emirates	47	205F	236	240F
Yemen	15	21	22	21
ESCWA TOTAL	1456	2132	2231	2197
WORLD TOTAL	2574	3753	4036	3883
ESCWA percentage of world total	57%	57%	55%	57%

Source: FAO Yearbook Production 1994 (Rome, FAO, 1995), vol. 48, table 66.

Note: F=FAO estimate.

C. FUTURE DEVELOPMENTS

Although often overshadowed by the reports on the promise of genetic engineering, the future prospects of plant cell and tissue culture technology are by no means less spectacular. Major developments in this technology take place within the field of cell cultures to provide an alternative to seeds or micropropagation. It is probably only a matter of time before the performance of a number of liquid plant cell culture systems will be improved to the point where they can be combined with bioreactor technology for economic, large-scale plantlet production.

It is generally accepted, however, that much higher production efficiency must be achieved before somatic embryos can compete with the seeds of most species on a commercial basis (38c). Crops that rely on vegetative propagation (such as tubers, banana, pineapple, coffee, palms) would particularly benefit from liquid cell culture systems for providing planting material. For at least one plantation species, oil palm (*Elaeis guineensis*), the large-scale production of somatic embryos to provide clonal material is already a reality.

An alternative promising application of liquid plant cell cultures is the production of secondary metabolites. Although this application area is still in its infancy, it seems more promising to establish plant cell lines capable of producing vitamins, pigments, enzymes, flavors, pharmaceutical and food additives than to attempt to engineer the genes that control the synthesis of these compounds into yeast or bacteria and subsequent mass-scale production of the compounds using bioreactor technology.

In order to produce economically secondary metabolites using PCTC, the production level of the desired product must be increased. Plant cells only produce small amounts of secondary metabolites. Therefore, there is first a need to establish tissue culture systems that synthesize the desired product at very high levels. Secondly, there is a need for bioreactor systems that can manipulate the genetic and biochemical capabilities of plant cell cultures to raise production levels even higher.

Only a very limited number of secondary metabolites is currently manufactured on a commercial scale. Since 1983, the Japanese company Mitsui Petrochemical Industries produces shikonin, used both as a dye and a pharmaceutical (anti-inflammatory and antibacterial) from *Lithospermum erythrorhizon* cell suspension cultures. In 1988 the estimated annual market value of shikonin was about US$ 600,000 - far below the US$ 20 million to US$ 50 million invested in the original research and development work. The final cost of the product, however, fell to US$ 4,000 per kg which compares favorably with US$ 4,500 per kg for the substance extracted from the roots of *L. erythrorhizon*. It should be noted that Kanebo, a Japanese cosmetics corporation which developed lipsticks containing shikonin, realized a turnover of about US$65 million in two years through the sale of 5 million lipsticks (84a).

Similar to the production of shikonin, other pharmaceutical raw materials, such as berberine and ginseng are produced from roots of *Coptis japonica* and *Panax ginseng* About 25% prescription medicines and various raw materials are obtained from plants and natural sources for the manufacture of these products, which are not enough to meet the consumers' demand. Table 15 indicates secondary metabolites for which liquid plant cell cultures could provide profitable alternatives for production.

Obviously there are some prospects to explore plant cell culture production of secondary metabolites. In contrast to considerable investments by Japanese companies in research on liquid plant cell cultures (the Japanese market for food additives is approximately 450 billion yen annually (84d)), few North American and European companies have been enthusiastic about the prospects for profitable industrial production of plant metabolites. There are reports, however, on Canadian and Israeli ventures into the commercial exploitation of anthocyanin (food additive: colourant) by cell culture technology (139).

TABLE 15. THE WORLD MARKET VALUE FOR SOME MAJOR SECONDARY PLANT METABOLITES IN 1987

Secondary metabolites	Annual needs	Industrial cost (US$ per kg)	Market value (in US$ million)
Pharmacy			
Ajmalicine	3-5 tons	1,500	4.5-7.5
Codeine	80-150 tons	650-900	52-135
Digoxin	6 tons	3,000	18
Diosgenin	200 tons	20-40	4-8
Vinblastine/vincristine	5-10 kg	5 million	25-50
Food-additives & fragrances			
Anthocyanins	n.a.	1,250 (139a)	n.a.
Jasmine oil	100 kg	5,000	0.5
Mint oil	3,000 kg	30	90
Natural vanillin	30 tons	2,500	75
Cosmetics			
Shikonin	150 kg	4,000	0.6

Sources: A. Sasson, *Biotechnology and Natural Products: Prospects for Commercial Production* (Nairobi, ACTS Press, African Centre for Technology Studies, 1992), p. 36: and G.A. Ravishankar and L.V. Venkataraman, "Role of plant cell culture in food biotechnology," *Plant Biotechnology, Commercial Prospects and Problems* (Andover, Hampshire, U.K., Intercept Ltd., 1993).

In most cases industrial production costs of secondary metabolites, using liquid plant cell cultures is still too high to warrant investments. It is estimated that plant cell cultures could only be profitable for plant substances with a value of US$80 per gram or more (84c). Several constraints could explain these high investment requirements. First, the time needed to select and stabilize cell lines is about two to three years: secondly, the lack of knowledge concerning biosynthetic pathways of secondary metabolites explains certain failures. Thirdly, it is difficult to extract the desired compounds, especially when the compounds accumulate as combined substances. Production by cell culture, therefore, remains only justified for rare products that are costly and difficult to obtain through other means.

D. DEVELOPMENTS IN SELECTED ESCWA MEMBER COUNTRIES

Within the ESCWA region, plant cell and tissue culture is the most well-established biotechnology in terms of the number of private companies, universities and government research bodies (see table 16). Egypt has the most experience in plant cell and tissue culture, and two major private companies have been active in mass propagation and export of potato tubers since 1990. The countries of the ESCWA region with the most extensive experience in plant cell and tissue culture techniques are Egypt, Jordan, the Syrian Arab Republic and Saudi Arabia. Developments in plant cell and tissue culture in these countries are highlighted below.

TABLE 16. NUMBER OF INSTITUTES OF ESCWA MEMBER COUNTRIES ACTIVE IN PLANT CELL AND TISSUE CULTURE IN 1996

ESCWA member country	Number of companies	Number of universities	Other*
Egypt	2 (90,91)	6 (5e)	4 (5e,5f,66,74,90,91)
Jordan	3 (5g,40,92,93)	3 (5g)	1 (5g)
Saudi Arabia (146a)	1	2	1
Syrian Arab Republic	1 (98)	1 (100)	3 (98,99,103)

Note: References in parentheses in this table refer to the corresponding references cited in full in the section on "References" in this study.

* Including public research institutes and foreign donor projects.

1. *Egypt*

Plant cell and tissue culture techniques have been fully mastered in Egypt, and cover a full range of plant tissue culture techniques (e.g. shoot organogenesis, somatic embryogenesis, *in vitro* pollination). Moreover, most Egyptian academic institutions have been involved in plant tissue culture over the last decade (see table 17).

Since 1990 private initiatives resulted in the routine use by two well-established firms (Danton [Lebanese ownership] and Picco [Egyptian]) of basic tissue culture techniques for mass propagation of potato, banana, strawberry and ornamentals. Picco seems especially successful, with approximately US$ 2.5 million value of exports and 15,000 acres of land for fields, greenhouses and laboratory facilities (90,91). Picco has bought ELISA test kits from the Agricultural Genetic Engineering Research Institute (AGERI), located in Giza, Egypt, to test potato mini-tubers for the presence of viruses (66,74). For establishing their technological know-how for virus-free production of potato mini-tubers Picco consulted with the Plant Cell and Tissue Culture Department of the National Research Center (NRC) (91).

The Plant Cell and Tissue Culture Department of the NRC, in turn, benefited from an NARP project (National Agricultural Research Project financially supported by USAID and ASRT, 1990-94) for establishing virus-free production of potato seeds through tissue culture techniques. The NARP project provided both training and equipment. The Plant Cell and Tissue Culture Department of the NRC also started exploring commercial opportunities in 1995 by selling micropropagated banana, mulberry and ornamental plantlets. This adds approximately US$ 3,000 to the department's yearly budget of US$ 14,700 (91).

AGERI, the most established institute working on genetic engineering of plants in the region, has also developed extensive tissue culture protocols for regenerating transformed plant tissue. For instance, AGERI developed tissue culture protocols for tomatoes and potatoes to support genetic engineering of plants (66, 74). For regeneration purposes, AGERI also aims to develop tissue culture protocols for cotton, faba bean and maize (72d).

As part of a collaboration with ICARDA, AGERI received US$ 30,000 (104c) in 1993 to establish a wide cross between short- and long- season varieties of lentil, using embryo rescue (103b). Difficulties in the synchronization of flowering of the lentil varieties limited the chance to obtain sufficient calli for establishing the wide cross (104c).

TABLE 17. EGYPTIAN INSTITUTIONS USING PLANT CELL AND TISSUE CULTURE TECHNIQUES FOR CROP IMPROVEMENT

Institute	Activities started in:	Main activities
1. Assiut University, Dept. of Horticulture (5e).	1984	Research: • Tissue culture used for selecting salt and/or drought-tolerant garlic species.
2. National Research Center, Dept. of Plant Cell and Tissue Culture (90,91).	1985	Research: • Tissue culture artichoke; production medical compounds, selection for salt tolerance. • Cell and tissue culture of maize, sweet potato, pea, bean. • NARP sponsored (USAID and ASRT) tissue culture project for mass-micropropagation of virus-free potato seeds. Commercial Services: • Mass-micropropagation of banana, mulberry and ornamentals.
3. Rice Research and Training Complex, Sakha (5f).	1986	Research: • Anther culture of rice to allow crossings of *japonica* and *indica*. • somaclonal variation, protoplast fusion to develop salt- and cold- tolerant varieties.
4. Date-Palm Production Improvement Programme, Aswan (5e).	1988-1992	Research: • Tissue culture for mass-micropropagation of date palm.
5. Agricultural Research Center, AGERI (66,74)	1989	Research: • micropropagation protocols for potato and tomato.
6. Danton (90,91)	1990	Commercial Services: • Commercial production of virus-free potato seedlings.
7. Picco (90, 91)	1990	Commercial Services: • Commercial production of virus-free potato seedlings. • Mass-micropropagation of banana, strawberry and ornamentals.
8. Zagazig University, Dept. of Horticulture (5e).	*	Research: • Mass-micropropagation of ornamentals, tree species. • Tissue culture for the development of salt- and drought-tolerant, and virus-free plants.
9. Ain Shams University (5e).	*	Research: • Mass-micropropagation of date palm, strawberry, asparagus and artichoke.
10. Al Azhar University (5e).	*	Research: • Anther- and ovule- culture for improvement of maize, rice and sorghum.
11. Alexandria University (5e).	*	Research: • Protoplast culture and fusion of flax.
12. American University (5e).	*	Research: • Tissue culture of vegetables and tree crops.

Note: References in parentheses in this table refer to the corresponding references cited in full in the section on "References" in this study.

* No data available.

2. *Jordan*

Jordan's experiences and achievements in PCTC are mainly geared towards micropropagation and have been established over the last five to seven years (see table 18).

TABLE 18. JORDANIAN INSTITUTIONS WORKING ON RESEARCH AND APPLICATIONS OF PLANT CELL AND TISSUE CULTURE

Institution	Financial resources	Activities started in:	Main activities:
Private Company: Celtis Labs (5g,40).	• Sukhtian Company. • Revenues from sales.	1989	Commercial Production: • Micropropagation of ornamental plants. Technology Implementation*: • Micropropagation of virus-free potato mini-tubers; • micropropagation of banana seedlings.
Public Research: Plant Production Department, Faculty of Agriculture, University of Jordan (5g).	• University of Jordan. • CIDA**.	1989	Training and Research: • Micropropagation protocols for strawberry, potato, ornamental plants, apple root stock and barley.
Public Research: National Center for Agricultural Research and Technology Transfer (NCARTT) (5g).	• Ministry of Agriculture. • France.	1991	Research: • Micropropagation of disease resistant apple rootstocks.
Public Research: Biotechnology Department, Center for Agricultural Research and Production - JUST (5g).	• JUST. • HCST. • Ministry of Agriculture. • IPGRI***	1992	Technology Development: • Micropropagation of virus-free potato mini-tubers. Research: • Tissue culture protocols for tomato, cucumber, almond, hawthorn, wild pear and batima.
Public Research: Mu'tah University (5g)	• Mu'tah University • HCST	1994	Research: • Micropropagation of virus-free potato mini-tubers.
Private Company: Ashruk Company (92).	n.a.	1994	Technology Implementation: • Micropropagation of virus-free potato mini-tubers.
Private Company: Jordan Kuwait Company for Agriculture and Food Products (93).	• USAID. • Revenues from sales of other product groups.	1995	Preparation R&D Activities: • Building of research and production facilities for micropropagation of apple rootstocks, potato, banana and ornamental cutflowers.

Note: References in parentheses in this table refer to the corresponding references cited in full in the section on "References" in this study.

* Technology Implementation: refers to efforts to transfer technology capacities into commercial production lines.
** Canadian International Development Agency.
*** International Plant Genetic Resources Institute.

Commercial activities and most research activities are aimed the production of virus-free potato mini-tubers. This technology is internationally well-established, cost-benefits have proved to be positive, initial investments are low and a short-term return on investments is guaranteed. According to international estimates, the use of virus-free potato seed can increase potato yields by 30% to 50%. The potato seed tuber market requirements for Jordan in 1993 were in the range of 9,500 tons with a market value of JD 3.2 million (126a). Of this total, 7.1 thousand tons of potato seed tubers were imported in 1993.

For the production of potato seeds from tissue culture propagated virus-free mini-tubers, one mini-tuber is required for the production of 216 seed-tubers. In favourable conditions (i.e., no virus contamination transferred by aphids), one mini-tuber can yield six-seed-tubers. Each seed can be propagated in the field in two subsequent cycles, yielding 216 seed-tubers. Therefore, the Jordanian market demand for mini-tubers amounts to approximately 44 tons.

The Jordanian institutions active in plant tissue culture do not share much interaction. Celtis Labs approached the Jordan University of Science and Technology to make use of its expertise on serological testing of viruses in potato mini-tubers. Financial barriers prevented any collaboration. Celtis Labs, however, did benefit from a training course at the University of Jordan, which was sponsored by the Canadian International Development Agency (CIDA). The Center for Agricultural Research and Productivity (CARP) of the Jordan University of Science and Technology (JUST) has initiated promising negotiations with a private company for the production of virus-free potato seeds. There is, however, no coordination of research and technology development activities in plant tissue culture at the national level.

Three companies are active in developing plant tissue culture activities for micropropagation purposes. The private drug and chemical Sukhtian Company is currently the most successful firm in plant cell and tissue culture, through its Celtis Labs enterprise. Protocols have been developed by the Celtis Labs company for many ornamental plants, and they are in the process of establishing large-scale production of virus-free potato tubers. This is hampered by the lack of a spectrophotometer to be used for virus control tests of germ plasm. Celtis Labs also intends to develop protocols for virus-free banana seedlings. In financial terms, Celtis Labs has achieved a return on investments since the company was established in 1989 (40). The General Manager of Celtis Labs obtained the necessary technological know-how by attending a one-year general course in the United States and by attending a course at the University of Jordan, sponsored by CIDA.

Another smaller company (Ashruk Co.) was established in 1994 and is working on developing potato and date palm micropropagation protocols. A major limitation is the lack of serological testing for the presence of viruses (92).

A third private company about to explore the opportunities of plant tissue culture is the Jordan Kuwait Company for Agriculture and Food Products established in 1995. In 1995 construction of buildings was started for a tissue culture laboratory to supply virus free seedlings to the company's orchard farm (e.g. apples, peaches) and for the production of potato mini-tubers, as well as virus-free banana seedlings and ornamental cutflowers. The Company received a loan from USAID for building the tissue culture facilities (93).

Research activities on PCTC are conducted by three Jordanian universities, mainly focusing on establishing micropropagation protocols. CARP has developed the most advanced plant tissue culture facilities in the country. The plant tissue culture laboratory (biotechnology department) was set up in 1992. In addition to the biotechnology department, CARP includes an animal husbandry and a crop breeding department. CARP is also responsible for land resources of the university. Available lands for crop research amount to 780 hectares (95).

The biotechnology department employs 10 professional staff members, of whom two are full-time professors. Initial financial support for the first three years (1992, 1993 and 1994) was jointly provided by the Higher Council for Science and Technology (HCST), the Ministry of Agriculture and JUST (95) (see table 19). The Ministry of Agriculture and HCST provided funding for equipment, chemicals and consumables, and JUST covered the cost of salaries and buildings for the centre. Since 1992 the annual budget of the Biotechnology Department decreased each year by an average 30%.

In 1995 research work focused on the production of vegetable seeds and seedlings via tissue culture for potato, tomato and cucumber. Other research work focusses on micropropagation and *in vitro* conservation of wild relatives of fruit trees in Jordan (i.e. bitter almond, hawthorn, wild pear and batima) (95). So far, research has mainly resulted in establishing tissue culture protocols for virus-free production of six potato cultivars (126b).

TABLE 19. FINANCIAL RESOURCES OF THE BIOTECHNOLOGY DEPARTMENT OF THE CENTER FOR AGRICULTURAL RESEARCH AND PRODUCTIVITY, JORDAN

Institution	1992	1993	1994	1995	TOTAL
JUST	30,000	30,000	20,000	20,000	100,000
Ministry of Agriculture	25,000	12,500	12,500	-	50,000
HCST	15,000	7,000	7,000	-	29,000
IPGRI	-	-	-	7,000	7,000
TOTAL annual budget	70,000	49,500	39,500	27,000	186,000

Sources: M.A. Al-Assaf Researcher, Agriculture and Water Sector, Higher Council for Science and Technology, Jordan, Personal Communication, 1995/96; and R.A. Shibli, *Plant Tissue Culture in Jordan*, Biotechnology Center, Jordan University of Science and Technology, Irbid, Jordan, 1995.

The Department could test the quality of the potato tubers at different stages for the presence of five different viruses, using ELISA (Enzyme Linked Immunosorbent Assay). In 1995 the biotechnology centre of JUST completed all stages for the production of virus-free potatoes and planned to serve seed companies and farmers by the end of 1995 (92). The capacities of the biotechnology department for the production of mini-tubers amounts to approximately 7% of the Jordanian market demand, taking into account favourable conditions for the vegetative propagation of mini-tubers into seed-tubers. In the course of 1996, negotiations between a private company and JUST started for the production of virus-free potato seed-tubers. There are two options for cooperation between the private company and JUST:

(a) Risk and interest sharing of the final product. This implies JUST would have to monitor the quality (percentage of virus-free seed-tubers) of the entire process.

(b) JUST would only bear responsibility for the production of mini-tubers. The private company would pay production costs and provide JUST with a profit margin for the mini-tubers.

Both parties are in favour of option (b).

In 1989 a five-year collaboration started between McGill University in Canada and the Faculty of Agriculture, University of Jordan, for establishing research capacity in agrobiotechnologies through training programmes (i.e. animal embryo transfer and semen-processing, plant tissue culture, fermentation and enzyme technology). The project budget totalled US$ 2.1 million of which US$ 770,000 was funded through CIDA, US$ 192,000 by McGill University and US$ 1.2 million was contributed by other non-government sources (University of Jordan). Of this total amount, 31.2% was spent in Canada and 68.8% was spent in Jordan (i.e. US$ 1.5 million) (96a).

Through this collaborative programme, approximately US$ 80,000 (92) were spent on equipment for a small tissue culture laboratory with a greenhouse at the Plant Production Department, Faculty of

Agriculture. Research is conducted on micropropagation protocols for strawberry, potato, ornamental plants, apple root stock and barley. So far the main output is restricted to training of B.Sc. students (92). Indirectly, Celtis Labs benefited considerably from the CIDA project, as the main initiator of Celtis Labs attended training courses on plant tissue culture at the University of Jordan.

At the National Center for Agricultural Research & Technology Transfer (NCARTT) of the Jordanian Ministry of Agriculture, a small modern tissue culture laboratory was established in 1991 with French cooperation. Its staff comprises two agricultural engineers trained in tissue culture and two support staff. The main activity comprises the micropropagation of disease-resistant apple rootstocks. In 1992, 3,000 seedling trees were in various stages of greenhouse and field development (5g). Some virus-free rootstocks were obtained in 1995 and are being prepared for grafting and further testing and evaluation (95).

Mu'tah University (Faculty of Science) established a modest plant tissue culture laboratory in 1994, jointly supported by HCST. Tissue culture research specifically focuses on the production of virus free potato mini-tubers (94). Up till 1995, the research was still at the *in vitro* propagation stage (95).

3. *Saudi Arabia*

In Saudi Arabia, the King Abdulaziz City for Science and Technology (KACST) is the leading Government institution for supporting applied scientific research as well as for coordinating the activities of Saudi Arabian scientific research institutions. Financial support made available by KACST for scientific research in Saudi Arabian institutions amounted to a total of SRls 400 million for the period 1978-1992 (148a).

Through the grant programme of KACST, two basic research initiatives in plant tissue culture were sponsored for three years at King Saud University in Riyadh. One project conducted by the plant protection department of King Saud University focused on the serological typing of potato virus diseases, using ELISA and the subsequent development of a tissue culture protocol for producing virus-free potato mini-tubers. The total grant from KACST amounted to SRls 883,375 (149a). The second project is still ongoing and is concerned with induction and selection of salt and drought-tolerant lines of wheat by plant tissue culture. Total grant money amounts to SRls 625,000 (149b). An overview of all plant tissue culture activities in Saudi Arabia is indicated in table 20.

As can be seen from table 20, most plant tissue culture activities in Saudi Arabia are geared towards date palm. Even some private commercial activities materialized in recent years. Because date palm is difficult to multiply, tissue culture forms a good alternative to traditional offshoot propagation.

The first activities of date palm tissue culture in Saudi Arabia date back to 1982, when the Date Palm Research Center (DPRC) was established at King Faisal University in Hofuf. By 1986 the Center had developed a protocol for somatic embryogenesis and in 1995 the Center established a protocol for organogenesis, which is the preferred method for commercial micropropagation (154).

More important, DPRC conducted a long-term experiment comparing the performance of tissue culture derived date palm plantlets from the United States, the United Kingdom and France with that of traditional offshoots. It was established that tissue culture plantlets grow faster and therefore reach the fruiting stage at an earlier age. The flavour-quality of dates is similar in comparing both methods. Of all public research institutes active in tissue culture of date palm, DPRC suffers most from a lack of continuity in research activities. Since 1986, the senior research staff in tissue culture have been expatriates. No attempts at mass-scale micropropagation of date palm have been made because human resources and the

budget are not sufficient and because the mandate of the administrating King Faisal University is primarily for education and basic scientific research (146a).

TABLE 20. INSTITUTIONS IN SAUDI ARABIA WORKING ON RESEARCH AND APPLICATIONS OF PLANT TISSUE CULTURE

Institution	Financial resources	Activities started in:	Main activities:
Public Research: Date Palm Research Center Hofuf	• King Faisal University	1982	Training and Research: • Development of 2 tissue culture protocols for date palm. • Field trials of tissue culture date palms. • Hosted three national symposiums on date palm.
Public Research: Plant Production Department King Saud University - Riyadh	• KACST *. • King Saud University.	1983	Training and Research: • Development of tissue culture protocols for arak (*Salvadora persica*), date palm, pomegranate, potato, and strawberries.
Public Research: National Agricultural and Water Research Center - Riyadh.	• Ministry of Agriculture. • Revenue from sales	1989	Research: • Development of a tissue culture protocol for date palm. Technology Development: • Micropropagation of date palm. Commercial Production: • Sale of micropropagated date palms to farmers.
Public Research: Plant Protection Department King Saud University - Riyadh	• KACST. • King Saud University.	1989	Research: • Serological typing of potato virus diseases using ELISA. • Development of a tissue culture protocol for potato. • Induction of drought/salt tolerant wheat lines.
Private Company: Saudi American Plant Development - Dammam	• Escagenetics Co. (USA) • Revenue from sales	1992	Research: • Development of 2 tissue culture protocols for date palm • Field trials of tissue culture date palms. Technology Development: • Micropropagation of date palm. Commercial Production: • Sale of micropropagated date palms to farmers.

Sources: ESCWA, Mission Report on Date Palm Micropropagation in Saudi Arabia, 19-24 October 1996, p. 34; A.A. Al-Baiz, National Agriculture and Water Research Center, Riyadh, Saudi Arabia, Personal Communication, 1996; A.J. Boumarah, General Manager, Saudi American Plant Development Company, Dammam, Saudi Arabia, Fax Communication, October 1996; K. Al-Maarri, King Faisal University, Hofuf, Saudi Arabia, Personal Communication, 1996; and I.M. Al-Shahwan, Associate Professor of Plant Pathology, Plant Protection Department, Faculty of Agriculture, King Saud University, Riyadh, Personal Communication, 1996.

In 1986, prior to the establishment of a tissue culture laboratory by the Ministry of Agriculture, a group of private local and international investors expressed interest in developing date palm tissue culture protocols for the commercial production of date palm plantlets (159). More than US$ 100,000 was invested in the development of a protocol by an American research company. Although the protocol was successfully developed, the Ministry of Agriculture decided to establish its own tissue culture laboratory for the development and production of date palm plantlets by tissue culture.

The Date Palm Tissue Culture Laboratory was established in 1989 with the National Agricultural and Water Research Center of the Ministry of Agriculture. The Laboratory has developed the most practical knowledge of large-scale date palm micropropagation in Saudi Arabia. In 1992, the Tissue Culture Laboratory started production, resulting in the mass propagation of approximately 19,300 plantlets in 1994 and 21,500 plantlets in 1995 (160, 161). A total of 15 varieties has been propagated by tissue culture, including superior varieties such as Nbootsafe, Nbootsultan and Sukhari. Plantlets are used for trials and a small amount is sold to farmers. High quality varieties of one-year-old varieties are sold for SRls 100 apiece whereas other varieties cost SRls 50. It is claimed that the institute utilizes both methods of date palm micropropagation (151). The majority of plantlets (75%) are produced by somatic embryogenesis. Since no comparative genetic analysis is made between the mother-plant and the clones, it is still to be established whether the mass-propagated plantlets will prove superior.

Recognizing the weaknesses of somatic embryogenesis, the Plant Tissue Culture Laboratory of the Department of Plant Production at King Saud University started working on developing shoot culture protocols in 1990 (154). Attempts are made to obtain plantlets from root-meristem tissue. In addition to its work on date palm, the Plant Tissue Culture Laboratory works on developing protocols for many other horticulture crops.

All public research institutes working on date palm indicated they have insufficient funds for chemicals and equipment (146). There is little communication between the different research groups and there is no coordination. Only the National Agricultural and Water Research Center of the Ministry of Agriculture has achieved mass-scale micropropagation of date palm. In 1990 the five-year plan (1990-1995) of Saudi Arabia put heavy emphasis on privatization (22a), opening the way for private initiatives in date palm tissue culture.

Current private initiatives in date palm micropropagation are limited to one company in Dammam. According to the general manager of the company—Saudi American Plant Development (SAPAD)—operations started in 1992 (152). SAPAD relies on micropropagation protocols of the Escagenetics corporation, an American public company. Both organogenesis and somatic embryogenesis protocols are used for commercial micropropagation. SAPAD could not report the use of DNA probes or protein analysis for testing the similarity of the plantlets to the original mother plant material. Escagenetics conducted field trials with tissue culture-propagated date palms in the United States and the Middle East for 12 years. Commercial sales of 10 superior date palm varieties began in September 1996. Price levels range between SRls 150 and SRls 500, depending on the variety and age of the plantlet (152).

The price levels of SAPAD for date palm plantlets can easily compete with current price levels of traditional offshoots. Nevertheless price levels of three-year-old offshoots of date palm dropped considerably over the last decade, from SRls 3,000 - SRls 6,000 in 1985 to SRls 500 - SRls 1,000 in 1996 (146). This is mainly due to increased production of offshoots and less demand from farmers.

A major obstacle for selling micropropagated plantlets is that farmers are not yet convinced of the superiority of tissue culture, as they do not trust the quality of plantlets. Overall the market for tissue culture date palm plantlets will remain limited as commercial agricultural firms in Saudi Arabia prefer crops which give a faster return on investments (162). This is further confirmed by the large quantities of dates that are donated by Saudi Arabia as foreign assistance (280,000 tons in the last 13 years) (166).

In this year, however, the Government has decided to destroy 850,000 trees infected by the red weevil. Although this mainly concerns old, non-productive trees, this will still increase the demand for date palm plantlets in the near future. Some wheat farmers are also changing to date palm cultivation as wheat subsidies have been drastically reduced over the last few years. For some years, the Government of Saudi

Arabia has restricted the import of date palm germ plasm because of fears about plant diseases. If companies such as SAPAD could increase their sale figures, this would result in reduced prices of date palm plantlets for tissue culture plantlets. This would make the technique a more favourable alternative to traditional offshoot propagation.

The mass-propagation of date palm is especially important for Saudi Arabia because it is a crop harnessing the water-holding capacity of arid soils. One of the genetic centres of origin of date palm can be found in the eastern province of Saudi Arabia (Hofuf area). The multitude of different varieties of date palms is highly appreciated by Saudi Arabian consumers and dates from elite varieties such as Nbootsafe, Rothana, Sukai, Sukhari, and Sultana fetch high prices.

4. *Syrian Arab Republic*

As in other ESCWA member countries, the core of the activities of the Syrian Arab Republic in biotechnology is for crop improvement. Biotechnology research and applications are basically restricted to tissue culture techniques. Table 21 summarizes organizations active in agricultural biotechnology in the Syrian Arab Republic.

TABLE 21. SYRIAN-BASED INSTITUTIONS USING BIOTECHNOLOGIES FOR CROP IMPROVEMENT SCHEMES

Institute	Started in	Main activities	Source of funding
1. The Atomic Energy Commission (99)	1982	Research • induce mutations, using τ radiation and chemical mutagens; • develop PCTC protocols for somatic embryogenesis as a prelude to genetic engineering; • attempts to use DNA molecular marker techniques for germ-plasm conservation and breeding programmes; • attempts to use DNA molecular marker techniques for studying crop insect pests.	Prime Ministry, IAEA, Atomic Commission of League of Arab States.
2. General Organization of Seed Multiplication (98,181a)	1987	Research • attempts to develop date palm micropropagation protocol. Commercial Services • development and sale of virus-free potato tubers.	Ministry of Agriculture.
3. International Centre for Agricultural Research in Dry Areas (54).	1992	Research • use of DNA molecular marker techniques for germ-plasm conservation and breeding programmes; • tissue culture techniques for breeding programmes.	International: through the CGIAR.
4. Arab Center for the Study of Arid Zones and Dry Lands (98)	1995	Research • set up a PCTC research unit.	Arab member States.
5. (-) Private Company (98)	1995	Commercial Services • micropropagation of ornamentals.	private

Note: References in parentheses in this table refer to the corresponding references cited in full in the section on "References" in this study.

Over the last few years, ICARDA (see chapter I) started work on tissue culture activities. Plant cell and tissue culture activities are mainly geared towards supporting breeding programmes enabling wide crosses, using embryo rescue (lentils) and ovule culture (chickpea). Research on producing doubled haploids of wheat and barley, using anther culture, started in 1988 and resulted in doubled haploid lines in bread wheat, of which 600 were tested in the field (54,103a).

Doubled haploids will both ease the identification of new molecular markers as well as increase the efficiency of breeding programmes. Cross-breeding time requirements can be significantly reduced as recessive traits can be more easily identified. The development of molecular markers for genome mapping as a supportive tool for plant breeding is an expensive long-term programme. Ultimately the use of molecular markers, when developed, saves time for breeding programmes and allows for identification of genotypes without the need for environmental selection criteria. Another application of double haploids, which is increasingly utilized internationally, is the release of double haploid lines as official varieties. The first ICARDA double haploid line was officially released in 1995 (167).

The most recent and only private initiative in plant tissue culture concerns a small company employing approximately 100 employees. Work started in 1995 on the micropropagation of ornamentals (98).

In 1987, a plant tissue culture laboratory was set up by the General Organization for Seed Multiplication (GOSM) furnished with locally manufactured equipment. The laboratory facilitates both research as well as micropropagation of virus-free potato shoots for large-scale production of potato seeds (i.e. tubers). Research on potatoes started in 1988 and mass-scale propagation of virus-free potato tubers first began in August 1995, when a new 1,000 m^2 greenhouse costing US$ 600,000, manufactured in the Netherlands, was built. The organization has additional greenhouses (5,000 m^2) and 15,000 m^2 of net-houses near Tartous to further propagate the virus-free potato tuber (98).

By the end of 1995 GOSM managed to produce 3% of the total Syrian requirements for virus-free tubers and hoped to meet the entire demand by the end of 1996. GOSM has an advantage that the organization is currently the only distributor in Syria for seeds of potato, cotton, wheat, barley, sugarbeet, maize, groundnut, chickpea, soybean, broad bean, lentil and pea. Moreover the organization has a yearly budget of US$ 90 million providing sufficient financial resources for research and seed multiplication. In 1990, GOSM initiated research on establishing date palm micropropagation protocols. The Syrian market requires 100,000 date palm seedlings yearly. So far no breakthrough in establishing suitable protocols for mass-scale propagation of date palm could be reported (98).

One of the leading scientists on date palm micropropagation in the Arab world, Khalil Al-Maarri, is affiliated with Damascus University and is presently working in Saudi Arabia at the Date Palm Research Center of King Faisal University in Hofuf. He has been responsible for developing a shoot tissue culture protocol over the last few years. He also is the author of the first book on date palm micropropagation in Arabic (154).

In 1982 the Agricultural Application Division of the Atomic Energy Commission of Syria (AECS) was set up for pest control and to support breeding programmes of the Ministry of Agriculture. Research crops include barley, garlic, wheat, soybean and olive. Initial research was mainly geared to induce mutations, using gamma radiation and chemical mutagens, which were then tested by the Ministry of Agriculture for superior traits (99).

Plant tissue culture has been used to improve the recovery of somatic mutations and subsequent embryogenesis. Five such mutated varieties of barley are now tested in greenhouses for improved drought and salt tolerance. The core of barley research is focused on the production of double haploids using anther culture which will speed up breeding programmes.

Similar research has been done on garlic for inducing fungal resistance and soybean to increase yield. AECS has furthermore developed strong links with ICARDA and has adopted similar research priorities. This includes attempts to produce double haploids of barley, using anther culture and the use of genetic

markers to support crop breeding programmes. In striking contrast to GOSM, most equipment is obtained from abroad through funding by foreign donors, such as the International Atomic Energy Agency (US$ 5,000 - US$ 6,000 yearly) and the Atomic Commission of the League of Arab States (US$ 2,000 yearly) (99).

E. Strategy considerations for building a commercial plant tissue culture industry

As noted in section B of this chapter, plant tissue culture appears to be the most likely candidate among the agrobiotechnologies for commercialization in the ESCWA region. Plant tissue culture offers many opportunities for the private sector and will continue to be an important scientific discipline. There are also some major disadvantages which militate against too high expectations for this sector in the ESCWA region. Both positive and negative prospects are indicated in table 22.

Table 22. Positive and negative indicators for prospects of the commercial plant tissue culture industry in the ESCWA region

Positive indicators.	Negative indicators
Short gestation period.	Volatile market with stiff competition and rapidly changing demands.
Low investment requirements.	Large-scale laboratories would reduce operating expenses considerably but require high investments.
Rapidly expanding world market demands.	High-value markets are difficult to access for ESCWA member countries.
Can replace high-value import of certified seeds (as with disease-free germ-plasm).	Quality control mechanisms are not available in ESCWA member countries.
Especially promising for vegetatively propagated tropical plant species (e.g banana, fruit trees, ornamentals, pineapple, palms, sugar cane).	• Traditional seed multiplication is still much cheaper with most plant species. • Low regional demand for ornamental plants; the biggest tissue culture market.
Tissue culture protocols are generally easily available.	Tissue culture protocols of especially plant species innate to the region (e.g. date palm, olive, pistachio, almond) are not yet available or difficult to obtain.

As can be seen from table 22, strategies for supporting commercial initiatives in plant tissue culture in any of the ESCWA member States must be carefully designed to avoid failure. Since any starting industry is vulnerable, protecting mechanisms should be set in place. The most obvious candidate for safeguarding any such industry is the national Government.

A second consideration for designing strategies is the identification of a proper set of targets to be achieved by building a commercial plant tissue culture sector. Such targets can be:

(a) To improve living standards of farmers and horticulturists by the provision of more high quality germ-plasm;

(b) To expand agricultural production by ensuring cheaper and more planting material;

(c) To reduce prices of agricultural commodities;

(d) To build training and research expertise in tissue culture;

(e) To increase the gross domestic product;

(f) To increase investment opportunities for the private sector.

Any strategy would become more sustainable if supported by several of the above-mentioned objectives. If plant tissue culture can realize target (a), the farming community will recognize the benefits of this technology and may become a powerful ally of private investors interested in tissue culture (target f) and vice versa.

Objectives (b) to (e) are more of strategic importance to the national interest of countries concerned and are not necessarily of interest to target groups. For instance, in the case of target (b), the availability of more and cheaper planting material may well reduce input requirements for farmers but if food commodity prices also drop as a consequence of more available crop produce, farmers do not necessarily stand to benefit.

The public research community may benefit in the short term if target (d) is chosen as the main objective. But if research outcomes are not made available to the private sector nor practical for farmers to use, the technology will soon be lost to the country.

It stands to reason that any country strategy aiming to create a commercial plant tissue culture industry, be it public or private, must mobilize specific interest groups. Since tissue culture plantlets are perishable and have to fulfil high demands in a short time-span (i.e. planting season), the retail sector usually does not play a major role in this sector. The exception is the production of ornamental and flowering plants for which demand and production are steady throughout the year.

Apart from biotechnologies for ornamental and flowering plants, the most likely candidates to be targeted for supporting a commercial plant tissue culture industry are farmers, private investors and agricultural companies. Special considerations for developing commercial plant tissue culture services in view of such target groups are reviewed below.

1. *Plant tissue culture for farmers*

A major challenge for boosting plant tissue culture initiatives in the ESCWA region is to mobilize the critical interest of farmers. In most countries of the Middle East, the agriculture sector is marginalized in comparison with other sectors (e.g. construction, energy, health, defence, water). Food commodities are heavily subsidized and farmers are not united in associations or corporations.

Therefore, one of the immediate objectives should be to unite or to allow farmers to become a critical demand-driven consumer group which will be motivated to purchase superior planting material (i.e. tissue culture germ-plasm). A critical mass of farmers will provide continuous incentives to tissue culture laboratories to improve and diversify their production.

In choosing this strategy, the tissue culture laboratories must be protected against sharp changes in market demand. In case of the involvement of the private sector, special start-up Government subsidies or public-private joint ventures may be considered. In addition, a system whereby a national certificate is issued indicating the quality of production can be developed, certified and monitored by public research bodies to protect both farmers and companies.

An alternative strategy with better development expectations in the short run is to channel tissue culture products into the existing marketing channels of seed and planting material. In the Arab world, such marketing channels are often centrally coordinated by a Government body and prices are fixed. Farmers are generally obliged to obtain tissue culture plantlets in the absence of alternatives. This strategy is, of course, very beneficial for any commercial tissue culture initiative. Farmers may not directly notice any benefits as they do not have an alternative to obtain seedling material. Although this option may sound somewhat drastic, it can ensure the protection and building of a strong commercially oriented tissue culture sector. Once a sound commercial seed development industry is established, the distribution of planting material can be decentralized, opening the market for competition and thus serving the interest of farmers. Of course, this kind of general strategy cannot be directly applicable to all individual situations of the individual member countries. For this reason, more specific strategies are needed and will be further reviewed in chapter IV.

2. *Private sector opportunities for plant tissue culture*

In view of the concerns of private investors in developing countries, special care should be taken to stimulate interest by this sector in plant tissue culture. Both investors and new biotechnology entrepreneurs face severe financial constraints in developing countries.

New private biotechnology companies will first have to deal with obtaining capital. In most developing countries, there are no private-venture capital companies that could help to supply equity capital (168d). Share markets are frequently insufficiently developed or are limited to traditional well-established firms, rendering access to equity for new firms in developing countries difficult.

Over the last decade, the public finance situation has worsened in most developing countries as a result of economic restructuring programmes. Public expenditure has been drastically reduced and projects with long gestation periods have been cancelled. This is especially damaging for biotechnology as long periods for return on investment are the rule rather than the exception. Moreover, the private sector in developing countries had to operate with extremely high interest rates in real terms during the 1980s. This also reduced the interest of international biotechnology companies in investing in developing countries (168d).

In most restructuring programmes, developing countries are awarding high priorities to privatization. The private sector, however, is not sufficiently equipped to simply replace or expand operations of public companies and institutions. Increasingly, good governance is recognized as the most suitable approach to warrant sustainable development at the country level (169). Privatization then becomes one of the most powerful tools available to Governments to stimulate and harness the development of new products and investments. As can be seen from the above-mentioned financial constraints on entrepreneurs interested in biotechnology, the private sector in developing countries must be safeguarded by the Government in order to be useful in exploring commercial plant tissue culture opportunities. The support of the Government for the private sector should not be overprotective but rather client-oriented.

The protection of newly established private tissue culture laboratories is especially important as the market for micropropagated plants is "inelastic" in nature (i.e. marketability is directly linked to the prevailing economic conditions) (124d). The market for field crops is seasonal, demanding a voluminous supply of plants within a short time-span. In addition, plant germ-plasm is vulnerable capital material when it comes to transport and storage. The Government should step in to allow for special facilitated import and export regulations to avoid losses. Once the private sector is firmly established, Governments can reduce their protective measures and allow for more competition, thereby ensuring a transition to a full market economy.

A possible government strategy may focus on establishing a national tissue culture laboratory with the main purpose of developing tissue culture protocols for a wide range of plant species of interest to

agricultural producers, horticulturists and foresters. The foremost target group of such an institute should be private companies. Private companies should be enabled to purchase protocols or improved germ-plasm for mass propagation. In Morocco, such a successful division of tasks was realized between the public research Institut National de Recherche Agronomique and the private company Domaines Agricoles, resulting in the large-scale micropropagation and distribution of 170,000 date palm plantlets (131).

In addition, advisory services on tissue culture technology supplied by a public tissue culture laboratory could encourage private companies to set up a plant tissue culture company for commercial purposes. This important link between public research bodies and the private sector seems lacking in most ESCWA member countries at present. In Jordan, government officials (i.e. university researchers) are not allowed to indulge in private initiatives (39). In Egypt there are numerous public research bodies in biotechnologies with overlapping research areas. However, hardly any private biotechnology initiatives have been made in Egypt, nor in any of the other ESCWA member countries.

In sharp contrast, the United States, the most successful country in biotechnologies at present, has, from the outset, adopted a key policy of facilitating the commercialization of research results and the transfer of technology between the American public and private sectors. The federal support levels in the United States for biotechnology research are still increasing (see box 1). This positive climate for biotechnology is supported through close links between universities and industry and also by the large availability of venture capital for the establishment of small companies.

Thanks to the strong relationships promoted by United States Government policies between public research bodies and biotechnology industries, a spontaneous diffusion of innovations from universities to private companies is taking place. Critics complain that such strategies have resulted in overcharging development costs to consumers. When consumers buy biotechnology products, they first have to pay for the product itself as developed by the private company and, secondly, they contribute to the development costs by paying taxes to support the public research system. The close collaboration between universities and industry may also cause universities to lose their public role, a process which is rapidly progressing in the industrialized world.

Still, such strategies focusing on close collaboration between the public and private sectors are essential for the very nature of biotechnologies. It is difficult to draw sharp lines between basic research and technology development (170a). As a consequence, it will be the continued responsibility of public research bodies to develop and disseminate technologies with a high social pay-off, for which private companies are not capable or willing to take the financial risks. If public research leads to commercially promising technologies, the private sector can develop and disseminate technologies further.

The last observation is especially valid for plant tissue culture. Continuous basic research efforts are necessary as most of the revenue of a commercial laboratory is obtained through a few plant species of high value (124d). New introductions always fetch a premium. This calls for system development for new crops of high value in collaboration with plant breeders. In turn, this crucial combination requires either large private investments or close collaboration between public research and private companies. In developing countries, the latter option seems more realistic and would allow for more initiatives, thus diversifying and broadening the market.

III. STATUS OF BIOTECHNOLOGY POLICIES IN THE ESCWA REGION

Introduction

This chapter summarizes the activities of selected ESCWA member countries in the area of biotechnology. More detailed accounts of separate institutions can be found in previous chapters above. This section will pay special attention to government efforts concerning coordination and collaboration between biotechnology enterprises at the national level. The prime focus will be on agrobiotechnologies. Two biotechnologies are most commonly used in R&D to improve agricultural production. A more generic biotechnology is concerned with plant tissue culture (PTC) and a more advanced biotechnology is genetic engineering (GE).

Clearly, developments in biotechnology in Middle Eastern countries are not homogeneous but differ from country to country. The major differences between the four countries under consideration in the present study are indicated in table 23.

TABLE 23. STATUS OF BIOTECHNOLOGY DEVELOPMENT IN FOUR SELECTED ESCWA MEMBER COUNTRIES

Biotechnology parameters	Egypt	Jordan	Saudi Arabia	Syrian Arab Republic
Biotechnology policies	+	++	+	-
Genetic engineering research of plants	+++	+	+	+
PCTC research	++	++	+	++
Quality of human resources	+++	+++	++	+++
Access to international technology	+++	++	++	+
Commercial biotechnology services	++	+	+	+

Sources: ESCWA, "Inter-Agency Task Force Meeting, Biotechnology Applications and Technology Transfer, Survey of Biotechnology Activities in Egypt", mission report on visit to Egypt, 16-21 December 1995, ESCWA secretariat; ESCWA, mission report, visit to the Syrian Arab Republic, 3-7 March 1996; and ESCWA, mission report on date palm micropropagation in Saudi Arabia, 19-24 October 1996.

(-) absent; (+) initiated; (++) established; (+++) international competence.

Since biotechnology research has only been recently established in the region, the total number of biotechnology activities in each of the categories is limited. Therefore, table 23 does not provide an exact representation of the state of biotechnology in the related ESCWA member countries. The status of biotechnologies is likely to change rapidly and would not justify a comparison between the countries concerned as the sizes of populations and economies are distinct.

The most striking observation from the preliminary assessment of biotechnology parameters is the exceptional position of Egypt. Research in genetic engineering, access to international technology and commercial services are more advanced than in other surveyed countries. Only in the area of policy formulation and implementation is Egypt behind Jordan.

Government priority setting and coordination of R&D activities in biotechnology may be the most important instrument for employing biotechnology to its fullest potential. Optimal priority-setting will render R&D more sustainable when long-term developments of a national economy are taken into account. National coordination of research may ensure the optimal use of R&D in technology development and subsequent improvement in the production of capital goods and the provision of services. A more detailed description of factors related to national priority-setting in biotechnology of the individual countries is given below.

A. EGYPT

Egypt's human resources in biotechnology are both abundant and highly trained. In 1992 more than 4,000 scientists were active in the field many of whom had been trained in European and American universities (174a). This affects directly the quality of established GE and PTC research activities. There exist, however, large differences between research institutes concerning available resources. In addition, biotechnology research is not directly linked to other National Agricultural Research Institutes (NARI). This is reflected by the status of policies in this area: policy formulation for biotechnologies has just been initiated.

The Academy for Scientific Research and Technology (ASRT), through its National Egyptian Focal Point of Genetic Engineering and Biotechnology, is responsible for coordinating biotechnology research activities at the national level as well as providing linkage with regional and international institutions in biotechnology. Furthermore, ASRT is responsible for contributing to national planning of biotechnology activities. ASRT indicated that Egypt's strategy in biotechnology had been to initiate (176a):

> "... a more general biotechnology programme, to produce a critical intellectual mass of interacting scientists, and from this to produce the nuclei of competence capable of addressing the technological components of development problems."

For implementing this strategy, ASRT suggests that a national training and research institute for genetic engineering and biotechnology should be established to cover most sectors of biotechnology (agriculture, public health, environment and industry). According to ASRT, the agricultural sector is of primary concern, as most biotechnology research in Egypt is active in this sector.

ASRT has identified a broad range of biotechnology research areas of interest to Egypt, which are indicated in box 2. ASRT also promotes some biotechnology research projects through financial support. In all, the ASRT department for scientific and cultural relations has a budget of approximately US$ 50,000 per year to support and coordinate activities in biotechnology research out of a total budget of US$ 1.5 million (90). ASRT was founded in 1971 and until recently administered the National Research Centre together with eight specialized research councils.

In 1993 a National Biosafety Committee (NBC) was established by the Egyptian Government, which formulated a draft advisory policy titled: "The establishment of a national biosafety system in Egypt: regulations and guidelines" (179). With regard to intellectual property rights, Egypt is in the process of strengthening laws (formulated by ASRT) which would render medical drugs, pharmaceutical compounds, plant and animal species, and microbiological organisms as patentable subject-matter (180). In September 1996, the Egyptian Parliament approved the first official biotechnology strategy and work plan, which will take effect as from January 1997 (90).

With regard to regional activities, the ASRT acts as a focal point of the International Center for Genetic Engineering and Biotechnology (ICGEB). ICGEB was established by UNIDO in 1983 as an international biotechnology research institute. Since 1993 ICGEB has been an autonomous intergovernmental

organization for training and research in molecular and cell biology for the benefit of developing countries. In 1995, ICGEB consisted of 56 member countries.

BOX 2. IDENTIFIED PRIORITY BIOTECHNOLOGY RESEARCH AREAS IN EGYPT

AGRICULTURE

1-1-1 Construction of genomic maps for plants of economic value in Egypt.
1-1-2 Production of plants resistant to insect pests.
1-1-3 Production of plants resistant to bacterial and viral pathogens.
1-1-4 Production of plants resistant to environmental stresses.
1-1-5 Production of plants resistant to herbicides.
1-1-6 Development of plants with better production traits and nutritional content.
1-1-7 Improvement of Food Production.

ANIMAL AND FISHERIES PRODUCTION

2-1-1 Livestock - e.g. construction of genomic maps (cattle, buffaloes, sheep, camels).
2-1-2 Poultry - e.g. construction of genomic maps (chicken, rabbits, ducks, geese, pigeons).
2-3- Develop techniques: monoclonal antibodies, Polymerase chain reaction (PCR), genetic transformation.

INDUSTRY

2-1 Microbial biotechnologies.
2-1-1...production of bulk commodities (e.g. alcohol, acetic acid, fermented foods, solvents, baker's yeast, municipal liquid wastes).
2-1-2...production of high added value commodities (e.g. antibiotics, steroid drugs, amino acids etc.)
2-1-3...use of genetically engineered micro-organisms for large scale production of highly specialized products.

HUMAN HEALTH

2-1 Establish a national quality assurance system for diagnostics in Egypt.
2-2 Promotion of PCR applications.
2-3 Promotion of the use of transgenic animals in the medical research.

ENVIRONMENT

3-1-1 The use of bioindicators and biomonitors to detect pollution levels.
3-1-2 Bioremediation of pollutants.
3-1-3 Biodegradation and recycling of industrial and agricultural wastes.
3-1-4 Development of resistant plants to agropests and pathogens to minimize the use of chemical pesticides.
3-1-5 Development of biofertilizers to minimize the application of chemical fertilizers.

Source: Academy for Scientific Research and Technology (Egypt). The National Institute of Genetic Engineering and Biotechnology Project, Cairo, 1994.

Egypt also hosts regional offices of FAO and UNESCO, both of which act as donors for biotechnology training courses in the region and sponsored two training courses in 1995. Because of the current cash-flow problems of the United Nations, both organizations indicated that no commitments could be made for financing courses in 1996, as yet (182, 185). FAO-RNEA acts as the coordinating organization of the Near East Regional Inter-Agency Task Force on Biotechnology Applications and Technology transfer. The Task Force holds two meetings annually to inform the members of regional organizations that are active in biotechnology about its past and future activities.

The Microbiological Resources Center (MIRCEN) and the Inter-Islamic Network on Genetic Engineering and Biotechnology (INOGE), both members of the Inter-Agency Task Force, are active as regional biotechnology institutions and mainly focus on maintenance of a microbial culture collection (MIRCEN), conducting of training courses and the publication of newsletters. MIRCEN has held 14 training courses since 1981. ASRT hosts INOGE. Both organizations are dependent on extrabudgetary funds for holding training courses (provided by UNESCO, FAO, WHO, UNEP and ICARDA) (186, 187). The newsletters published by both institutions appear irregularly, depending on available donor funds.

B. JORDAN

Of the four countries surveyed, only in Jordan are specific biotechnology policies being implemented. A grant system for stimulating national biotechnology research provided a competitive environment in which several plant tissue culture initiatives (both commercial and research) emerged. Plant molecular biology research initiatives have yet to be established.

The first and second Arab conferences on biotechnology were held in Amman in 1989 and 1993 respectively. Both conferences were of a general nature, and reviewed biotechnologies ranging from genetic engineering to biotreatment of waste. The recommendations of the second conference for the ESCWA member States, with regard to the present status of biotechnologies, focused on (2a):

(a) Addressing specialized biotechnology activities (i.e. tissue culture, applications in the pharmaceutical and food industry, genetic engineering and legal issues related to biotechnology);

(b) Promoting investment in modern biotechnology.

By hosting both conferences, Jordan, and in particular its private sector, had a distinctive advantage over other ESCWA member countries with regard to promoting activities in biotechnology, making use of international and regional attention. As a result of the second conference Jordan included biotechnologies in its policy for science and technology (94). At the beginning of 1996, the total number of faculty member working on biotechnology at five of Jordan's public universities amounted to 38 out of 759 and the total estimated number of international publications was 37 (see table 24).

The Higher Council for Science and Technology (HCST) is responsible for the formulation of policies and strategies regarding biotechnologies in Jordan since 1992. The first National Science and Technology Policy was formulated in 1995. Biotechnology was not included as a separate chapter therein but was integrated with other priority areas. Emphasis was placed on biotechnologies with reference to water, land resources, environment and energy, as these areas constitute priorities within the policy of HCST. Final policy decisions are taken by the Ministry of Agriculture and the Ministry of Planning. More detailed information on policy formulation relating to biotechnologies is shown in box 3.

Before the Second Conference on Biotechnology in 1992, HCST was working to establish a National Biotechnology Center, responsible for all research and development in this field (5h). When a request for financial and technical support for establishing such a centre was turned down by the European Commission, HCST adjusted its strategy (94). Instead of focusing on centralized institution-building, which requires considerable investments for buildings, HCST is now focusing on stimulating R&D efforts by supporting promising initiatives at various institutions.

An important instrument of HCST in order to encourage selected biotechnology research groups is a special grant system. Total awarded biotechnology research grants amounted to approximately 200,000 Jordanian dinars (JD) for the period 1992 - 2000 (see table 25). These small projects, consisting of limited

financial support for several years, serve to improve the applicability of research findings in biotechnology, and to increase access to the technology by other private and public parties. After the conclusion of a project, researchers have to present their findings in reports as well as in meetings with interested parties, organized by HCST. HCST brings together the parties involved and thus serves primarily as a coordinator for supporting domestic technology transfer mechanisms (94).

TABLE 24. FACULTY MEMBERS OF FIVE JORDANIAN UNIVERSITIES ACTIVE IN BIOTECHNOLOGY AND PUBLISHING IN INTERNATIONAL PUBLICATIONS IN 1996

University	No. of faculty members	No. of faculty members in biotechnology areas	No. of international publications
Yarmouk University			
• Faculty of Science	148	7	5
Jordan University of Science and Technology			
• Faculty of Science	54	5	
• Faculty of Medicine	112	3	8
• Faculty of Agriculture	23	2	
University of Jordan			
• Faculty of Science	140	4	6
• Faculty of Medicine	121	8	2
• Faculty of Agriculture	74	7	15 (189)
Muta'h University			
• Faculty of Science	52	1	1
Al al-Bayt University	35	1	
• Faculty of Science			
TOTAL	759	38	37

Source: W. Owais, M. Al-Ajlouni and R. Shibli, "Jordan Country Study on Biological Diversity: Biotechnology," in UNEP Biodiversity Study in Jordan, Faculty of Science, Yarmouk University, Irbid, Jordan, 1996.

Note: References in parentheses in this table refer to the corresponding references cited in full in the section on "References" in this study.

The level of support varies according to the objective. Some research institutes receive somewhat larger financial support (e.g. JD 68,700) in order to establish a centre of excellence. Other institutes may receive a small grant (e.g. JD 2,000) to establish some research competence. This strategy of building a national biotechnology R&D framework aims at establishing R&D capacities within different already established research institutes, thereby avoiding additional administrative costs and investments in buildings.

HCST may also be involved in regional attempts to set up information and collaboration networks. The Arab League Educational, Cultural and Scientific Organization (ALECSO) is in the process of building a network with regional focal points. HCST may act as the regional focal point for biotechnology in Jordan. (94)

The coordinator of the Arab Biosciences Network is currently based in Jordan, at Yarmouk University in Irbid. The Network supports about four to five regional workshops on biosciences yearly, through donations of from UNESCO. The "biosciences" include all biology-related scientific disciplines and not biotechnology *per se* (190). As part of UNEP study on biological diversity perspectives for Jordan, the coordinator investigated the status of biotechnology in Jordan.

TABLE 25. LIST OF BIOTECHNOLOGY RESEARCH PROJECTS FUNDED BY THE HIGHER COUNCIL FOR SCIENCE AND TECHNOLOGY IN JORDAN

Principal Investigator	Institute	Title	Budget (JD)	Duration	Donor	Status
Rida A. Shibli Mohammad Ajlouni	Department of Biotechnology, Centre for Agricultural Research & Production, Faculty of Agriculture, Jordan University of Science and Technology.	Establishing a plant cell and tissue culture laboratory.	29,000	1993-1995	HCST Agriculture Sector	C
Ihsan Mahasneh	Faculty of Science, Muta'h University	(i) Establishing a plant cell and tissue culture laboratory. (ii) The Use of Algi as a Protein Source.	(i) 2,500 (ii) 2,000	1993-1994	HCST Agriculture Sector	C
Akel Mansour	University of Jordan	(i) Production of antisera for the identification of yellow-leaf virus in cucumber. (ii) Non-chemical methods to combat mosaic viruses in cucurbits.	(i) 9,360 (ii) 5,000	1993-1995 March 1995 March 1997	HCST Agriculture Sector	C O
Nizar Abu Harfeel	Biology Department, Faculty of Science, Jordan University of Science and Technology.	(i) The development of monoclonal antibodies. (ii) Development of pregnancy test kits.	(i) 14,500 (ii) 5,000	(i) 93-95 (ii) 95-96	HCST Agriculture Sector	C
Marwan Abdulwali	National Center for Agricultural Research and Technology Transfer (NCARTT)	Biological control of white fly[a/].	8,000	1994-1996	HCST Agriculture Sector	O
Farouk Quadan	ARCOMEX	Isolations of antigens against toxoplasma bacteria.	6,000	1995	HCST Industry Sector	C
	SUB-TOTAL		81,360	1992-1996	HCST	O
Malik S. Haddadin	Nutrition and Food Technology Department, Faculty of Agriculture, University of Jordan.	The Fermentation of Olive Pomace for the Production of Broiler Feed	10,000	1996-1997	HCST Agriculture Sector	O
Jamal Sawwan	Plant Production Department, Faculty of Agriculture, University of Jordan.	Developing early potato lines tolerant to viral diseases.	21,000	1996-1998	HCST Agriculture Sector	O
A.N. Al-Musa	• University of Jordan • NCARTT	Production of antisera specific to some plant viruses (tomato, potato, cucumber and squash).	68,700	1996-2000	HCST Agriculture Sector	O
Jamal R. Qasem	University of Jordan	Plants as natural sources of herbicides for broomrape (*Orobanche ramosa L.*)	13,000	1996-1999	HCST Agriculture Sector	O
	TOTAL		194,060	1992-2000	HCST	O

Source: M. A. Al-Assaf, Researcher, Agriculture and Water Sector, HCST, Personal Communication, Amman, 1995/96; and HCST, Earth Resources: Research and Development Projects, 1996-2000 (Amman, 1996).

Notes: Status: O = Ongoing; C = Completed.

a/ Biological control of white fly is not a biotechnology, by strict definition.

BOX 3. ELEMENTS OF THE JORDANIAN NATIONAL SCIENCE AND TECHNOLOGY POLICY DIRECTLY OR INDIRECTLY CONNECTED TO BIOTECHNOLOGIES*

28. i. Establishing and developing information bases for environmental matters, specifically on pollution and population, biodiversity, dangerous materials, communicable diseases, natural and water resources.

40. i. Supporting medicinal industry related research including that linked to the production of medicines and cures.

42. i. ... and developing animal health laboratories.
ii. Developing forestry, covering the survey of woods and the use of new technologies for their upkeep and firefighting as well as introducing suitable and economically profitable species.
iii. Developing crops necessary for food industries and animal products and canned agricultural products and developing and improving food industry equipment and machinery.

43. vi. Developing animal resources, covering increased productivity as well as setting correct standards and specifications and developing the hygiene of animal products; developing laboratories, Badia pastures and the sources of non-traditional feedstuff; studying animal/plant complementarity within the agricultural system.
vii. Undertaking studies and research for the development of plant production, covering domestic crop development and finding the suitable agricultural operations for various environmental zones and plant soils and raising soil fertility.
xi. Developing medicinal, aromatic and oil-bearing plants, as to their identification, classification, production and extraction of effective compounds.
xii. Developing flowers and decorative plants, and improving local wild plants and preserving them for the purpose of production at the commercial level.
xiii. Undertaking studies and research related to benefiting from crop residues to obtain animal feed or industrial materials.

48. ii. Documenting and protecting the natural environment, curbing decertification and assessing the environmental impact of exploiting natural resources.

49. xv. Undertaking comprehensive studies on the quantity and composition of household wastes, dangerous wastes and control and recycling methods.

52. iv. Utilizing new technologies in the analysis of food and medicine, for approving their quality and determining their expiry date.

54. ii. Developing new technologies, specifically genetic engineering, to improve the quality of animal and plant production.

55. vi. Transferring the agricultural technology capable of combating desertification, increasing plant resistance and intensifying afforestation.
vii. Developing traditional food industries through the introduction of new technologies.

57. ii. Utilizing new technologies for the purification of wastewater and recycling it according to existing international standards.

59. i. Utilizing technologies that are not harmful to the environment and utilizing reuse and recycling technologies to reduce waste.
iv. Utilizing modern technologies in waste dumps and exploiting biogas.
viii. Utilizing integrated (biological and chemical) means to combat agricultural pests and insects and producing high-resistance and high-immunity plant strains.
x. Utilizing modern technologies that assist in the recycling of industrial and agricultural wastes, or converting what is possible into beneficial materials.

Source: Higher Council for Science and Technology in Jordan, *National Science and Technology Policy* (Amman, 1995).

* Numbers for items refer to page numbers of the HCST policy document.

C. Saudi Arabia

As Egypt is on the brink of implementing its first biotechnology strategy and Jordan has been doing so since 1992, Saudi Arabia still has to formulate policy priorities for biotechnology at the country level.

Since its creation in 1977, the King Abdulaziz City for Science and Technology (KACST) has been the leading Saudi Arabian Government institution for supporting applied scientific research as well as for coordinating the activities of Saudi Arabian scientific research institutions. The main instrument of KACST in carrying out its mandate is the provision of grants to public research bodies through a number of programmes. Financial support made available by KACST for applied and national scientific research projects in Saudi institutions amounted to a total of 265 million Saudi Arabian riyals (SRIs) for the period 1978-1992 (148a). Table 26 indicates distribution of grants among the main scientific areas in which biotechnology research is used as a supportive element.

TABLE 26. LIST OF RESEARCH PROJECTS RELATING TO BIOTECHNOLOGY AND FUNDED BY KING ABDULAZIZ CITY FOR SCIENCE AND TECHNOLOGY, SAUDI ARABIA (1977-1993)

Research area	Number of projects	Budget (in Saudi Arabian riyals)
Health and medicine	11	24,511,409
Fermentation/bioreactor technology	9	17,488,712
Crop improvement	10	8,466,792
Livestock and fisheries	3	2,158,860
TOTAL	33	52,625,773

Source: KACST, General Directorate of Research Grants Programmes, Riyadh, 1994.

At the international level, KACST is charged with supporting joint-research programs between the Kingdom and international scientific institutions. In theory, this could be done either through providing grants for such programmes or through grants for conducting joint research activities.

KACST does not have a special grants programme for biotechnology. Rather, projects are selected on the basis of national needs assessment through the National Projects Program. Such national projects can have a budget allocation of up to SRIs 10 million. Other projects are based on recommendations from seminars or other government departments.

In the period from 1977 to 1993, KACST allocated more than SRIs 15 million to 14 specific biotechnology related projects. This amounts to approximately 5% of the total project research budget of KACST. Projects funded by KACST which are focusing on a biotechnology as their core research area are indicated in tables 26 and 27.

The lion's share (i.e. 65%) of KACST financial support for biotechnology research is in the areas of health and medicine. Agrobiotechnologies receive 12% of total research funds spent on biotechnology.

TABLE 27. LIST OF BIOTECHNOLOGY RESEARCH PROJECTS FUNDED BY KING ABDULAZIZ CITY FOR SCIENCE AND TECHNOLOGY, (1977-1993) (HEALTH AND MEDICINE)

No./Principal investigator	Title	Institution	Budget (SRls)	Time month	Status	Year funded
		Health and Medicine				
6/S.M. Al Rajeh	Molecular Basis of Inherited Neurological Diseases	National Guards Hospital	789,000	24	O	1993
2/N.R. Farid	Characterization, Regulation and Expression of the TSH Receptor	King Faisal Specialist Hospital	1,781,495	48	O	1992
10/A.S. Al-Tuwaijri	Studies on the Role of Cell-Mediated Immune Responses in the Pathogenesis of *Entamoeba Histolytica* Infections	King Saud University	556,000	30	C	1988
133/A.M. Ghandour	Studies on Some Aspects of the Epidemiology of Schistosomiasis in the Western Region of Saudi Arabia Using Immuno Diagnosis (ELISA)	King Abdulaziz University	458,000	24	C	1986
74/M.A. El-Hazmi	Aspects of Human Haemoglobin and Hemoglobinopathies in the Arabian Peninsula Studies at Genetic and Molecular Levels	King Saud University	6,473,900	60	C	1982
75/G.A. Jamjoon	Feasibility Study for the Automated Detection of Different Forms of the Malaria Parasites	King Saud University	88,100	12	C	1982
		Fermentation/Bioreactor Technology				
15/A.A. Abou-Zeid	Utilization of Saudi Dated and Their By-Products in Biosynthesis of Antibiotics	King Abdulaziz University	704,450	24	C	1987
86/A.N. Al-Rahman	The Potential Cultivation of Edible Fungi (e.g. Agaricus Bisporus and Podaxis Sp. in Saudi Arabia)	King Saud University	1,061,900	24	C	1983
15/A.H. Abu Zinda	Studies on the Production of microbial Protein from Hydrocarbon Source	King Saud University	995,000	48	C	1981
SUBTOTAL			12,907,845			

Source: KACST, General Directorate of Research Centre Programmes, Riyadh, 1994.

Notes: TSH = Thyroid-stimulating hormone. Status: O = Ongoing; C = Completed.

The funding role of KACST has obvious benefits. Through is various grant programmes, KACST contributes to promoting research initiatives in areas of national concern as well as encouraging talented researchers. However, the other side, overreliance on supplying grants to the research community as a tool for encouraging research and development may have resulted in ignoring other instruments for the promotion of R & D.

TABLE 28. LIST OF BIOTECHNOLOGY RESEARCH PROJECTS FUNDED BY KACST (CROP IMPROVEMENT AND LIVESTOCK)

No./Principal investigator	Title	Institution	Budget (SRls)	Time month	Status	Year funded
Crop Improvement						
61/M.N. Baraket	Induction and selection of salt and drought tolerant lines of wheat by plant tissue culture	King Saud University	625.000	36	O	1991
81/I.M. Al-Shahwan	Potato diseases and production of pathogen-free potato clones via tissue culture in Saudi Arabia	King Saud University	883,375	6	C	1989
77/Y.A. Al-Saheal	Gene effects in a di-allel cross of some local introduced wheats	King Saud University	212,400	24	C	1979
76/Y.A. Al-Saheal	Induced mutation of Saudi Arabian local variety of bread wheats (yield and yield components)	King Saud University	168,000	24	C	1979
Livestock						
71/E.M. Abu El Zein	Studies on some important arboviruses on animals in Saudi Arabia	King Faisal University	699,800	36	O	1991
TOTAL			15,496,420			

Source: KACST, General Directorate of Research Grants Programmes, Riyadh, 1994.

Notes: Status: O = Ongoing; C = Completed.

Next to its coordinative and supportive tasks, KACST is responsible for the formulation and implementation of national policies for science and technology development. KACST has the responsibility for building up scientific and technological capabilities in biotechnologies (5i). In December 1987, a national seminar in Riyadh on genetic engineering and biotechnology was sponsored by KACST and UNIDO, in collaboration with King Saud University. The seminar recommended the setting up of a Biotechnology Advisory Group (BAG) with the participation of governmental agencies, industry and universities. The objectives of BAG were outlined as follows (5i):

(a) Initiate and coordinate a biotechnology programme;
(b) Develop a biotechnology information base for Saudi Arabia;
(c) Organize training workshops on specific biotechnology aspects;
(d) Promote interaction between various disciplines and industry.

The first BAG meeting took place in November 1993. The meeting covered a multitude of scientific disciplines concerned with biotechnology. For the next meetings, it was decided to split the advisory group into smaller subgroups to address problems related to specific sectors (such as health, agriculture and bioreactor technology).

In 1995, a special Genetic Engineering and Biotechnology Program was initiated at KACST to determine research priorities and modalities for organizing biotechnology research. Some research, including molecular biology research, is planned to be carried out at KACST. Other preliminary plans are focusing on the establishment of a center for genetic engineering and biotechnology. (209)

D. Syrian Arab Republic

Of the four ESCWA member countries surveyed, the Syrian Arab Republic is the only country that does not have a coordinating body for biotechnology. As a consequence, no attempts at formulating biotechnology policies at the national level have been initiated (see table 23). This is characteristic for the way in which the Syrian Arab Republic has organized national authoritative coordinating responsibilities in agricultural research: no fewer than three ministries (the Ministry of Agriculture and Agrarian Reform, the Ministry of Economy and External Trade, and the Ministry of Irrigation) are involved in planning public agricultural research (192a).

This segregation of responsibilities is not necessarily without benefits. Individual research authorities which, are often streamlined according to crop commodities (e.g. cotton bureau, citrus bureau), can adjust priorities, rapidly creating the necessary flexibility to respond to problems in agriculture or opportunities in research. One such authoritative research body in the Syrian Arab Republic recently decided to explore the opportunities offered by biotechnology: the Atomic Energy Commission of Syria (AECS). The AECS is an integral unit of the Syrian Prime Ministry, which provides the commission with some autonomy (193).

On the basis of experience in biotechnology research, AECS organized an international biotechnology workshop in November 1996 to determine the strategic elements for developing long-term research programmes at AECS. International experts in various biotechnology disciplines (including human genome mapping, immunology and plant molecular biology, plant tissue culture) presented lectures and reviewed current research programmes of AECS. Owing to the lack of national formulated policies, AECS may promote national coordination of biotechnology activities in the country (196). Like ASRT and AGERI in Egypt, AECS is a focal point of the International Center for Genetic Engineering and Biotechnology (ICGEB).

The Syrian Arab Republic hosts the International Centre for Agricultural Research in Dry Areas (ICARDA), which was established in 1977. ICARDA is one of 18 international agricultural research centres, supported by the Consultative Group on International Agricultural Research (CGIAR), which is an international group of representatives of donor agencies, eminent agricultural scientists, and institutional administrators. The 1995 total research expenditures of ICARDA amounted to US$ 12.7 million out of a total budget of US$ 22.6 million (102a). The 1996 biotechnology budget forecast was estimated at US$ 530,800 (98); or approximately 4% of the total research budget is spent on biotechnology research.

Next to research, the biotechnology unit of ICARDA has a small-scale activity on familiarizing policy makers and research directors of national agricultural research centres with the prospects of DNA technology for crop improvement, through a yearly course on "DNA Molecular Marker Techniques". In future, more emphasis is to be expected on technology transfer aspects from ICARDA. A project proposal, to be funded by UNDP in the amount of US$ 3.5 million, will consider technology transfer as a core activity. The incentive for this proposal is the current lack of financial resources to enable all national agricultural research centers in the region to work on the same advanced techniques (54).

The Syrian Arab Republic is also the host country of the Arab Centre for the Study of Arid Zones and Dry Lands (ACSAD). ACSAD is an intergovernmental autonomous organization affiliated with the League of Arab States. Concerning biotechnology applications, ACSAD established a small tissue culture laboratory in 1995 with German technical assistance (99).

IV. RECOMMENDATIONS FOR ORGANIZING AGRICULTURAL BIOTECHNOLOGY RESEARCH

The above chapters reviewed biotechnology developments in selected ESCWA member countries; the crucial issue of this study: in what way can biotechnologies contribute most to agriculture? Which actors are involved and how can policies assist in bringing biotechnologies to benefit the countries of the region? It will first be necessary to identify the objectives. To quote M.S. Swaminathan, the former director of the International Rice Research Institute (199g):, "solving the problem rather than worshipping the tool should be the goal".

Section A below describes in what way technologies contribute to increasing agricultural production. Section B describes the importance of biotechnologies to developing countries and the need for technology transfer modalities to be in line with local technological capabilities. Finally, section C includes policy analysis and suggestions for Egypt, Jordan, Saudi Arabia and the Syrian Arab Republic.

A. Role of research in agricultural development

So far, the focus has been on biotechnologies in general. Now, the extent to which research may contribute to agricultural development *per se* in the ESCWA member countries will be explored. Science and technology, through investments in agricultural research, have made impressive contributions to the growth of the agricultural sector in many parts of the developing world. Since the mid-1960s, global food production has increased by 80%, with more than half of that increase in developing countries. From 1960 to 1994, yield increases in major food grains (maize, rice and wheat) nearly doubled (175a). Increases in productivity as measured in terms of kilograms per hectare can be attributed to the following:

(a) Improved crop varieties;

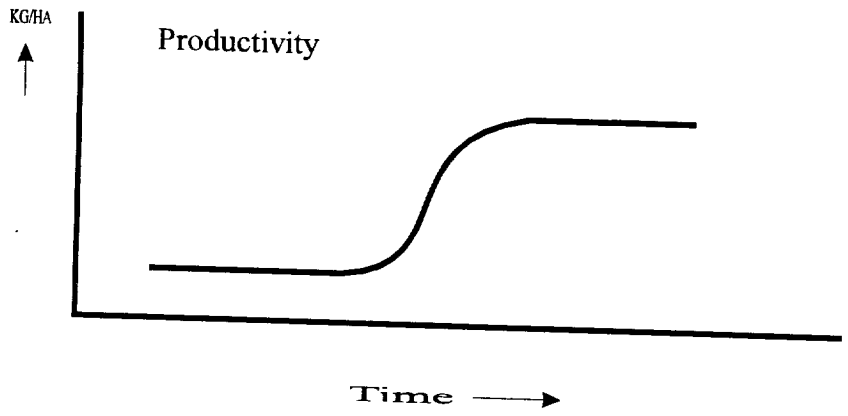
(b) Improved control of environmental factors (through irrigation, fertilizers and improved crop and resource management technologies).

The impact of biotechnologies is mainly expected at the first level. How can biotechnologies contribute to the improvement of crop varieties? Progress in crop improvement research can be described in three stages. The first stage is organismic plant breeding, which is traditional plant breeding using intact plants. The progeny are referred to as hybrid plants. Hybrids accounted for the main increase in agricultural productivity over the past four decades. The second stage is cellular plant breeding, using cell- or tissue-culture techniques for crop improvement. The related progeny are so-called "somatic" (asexual) hybrids. Finally, molecular plant breeding, or genetic transformation, is the third stage. The Progeny are referred to as transgenic plants (21a).

Taking the contribution of one selected technology to agricultural productivity will result in a sigmoidal impact curve over time, as indicated in figure III. Initial development of the scientific breakthroughs in a technology will occur slowly as the many other actors in the technology development chain contribute improvements. Finally, the technology will be fully developed, thereby reaching a plateau value for its potential contribution to agricultural productivity. The same trend occurs when agricultural producers start utilizing the technology. Initial adaptations will only be made by some farmers. After some time, the majority of agricultural producers will follow suit, and the adaptation curve will again level off to a maximum when late adapters follow suit. In many of the industrialized countries, the impact of hybrids is ensuring a steady increase of productivity. Before the impact of hybrids levels off to reach its plateau value, it is likely that tissue culture and genetic engineering technologies will ensure a continuous increase of productivity, as indicated in figure IV.

it is likely that tissue culture and genetic engineering technologies will ensure a continuous increase of productivity, as indicated in figure IV.

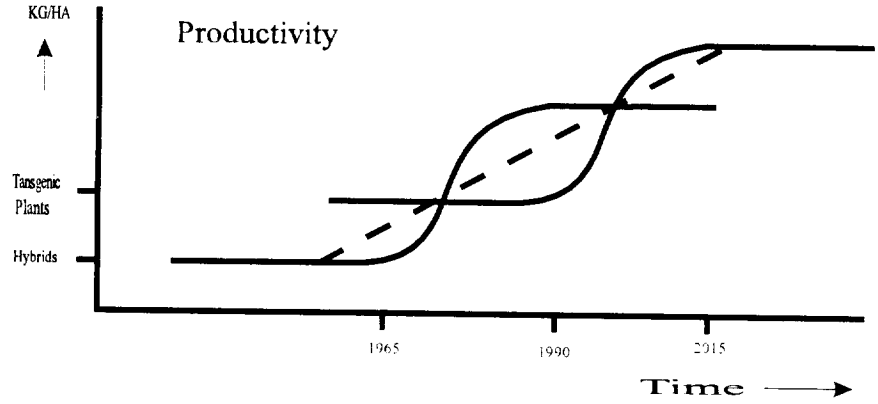
Figure III. Impact of a successful technology on agricultural productivity over time



Developing countries for which agricultural productivity is considerably lower may gain more by focusing on generic technologies aimed at improving varieties and at improving control of environmental factors. This is, for instance, the case with wheat cultivation in the Syrian Arab Republic and Jordan (see figure V-3). The Netherlands have the highest agricultural productivity in the world. The gap in productivity between the highest productivity rates and those of the Syrian Arab Republic and Jordan indicates the potential to make use of existing or generic technologies to increase productivity. Egypt and Saudi Arabia show a much more favourable time trend increase in wheat productivity. By comparison, the United States has only half the wheat productivity of the latter two countries. Productivity in wheat is directly correlated with timely access to water. Improvement in wheat productivity depends mainly on better access to water or improved drought tolerance. Both Egypt and Saudi Arabia grow wheat under irrigation, the former from the Nile delta and the latter by pumping water from aquifers. In any case, both Egypt and Saudi Arabia need to utilize more advanced technologies to increase wheat productivity further. It is noteworthy that the Syrian Arab Republic also managed to achieve higher yields than the United States in recent years.

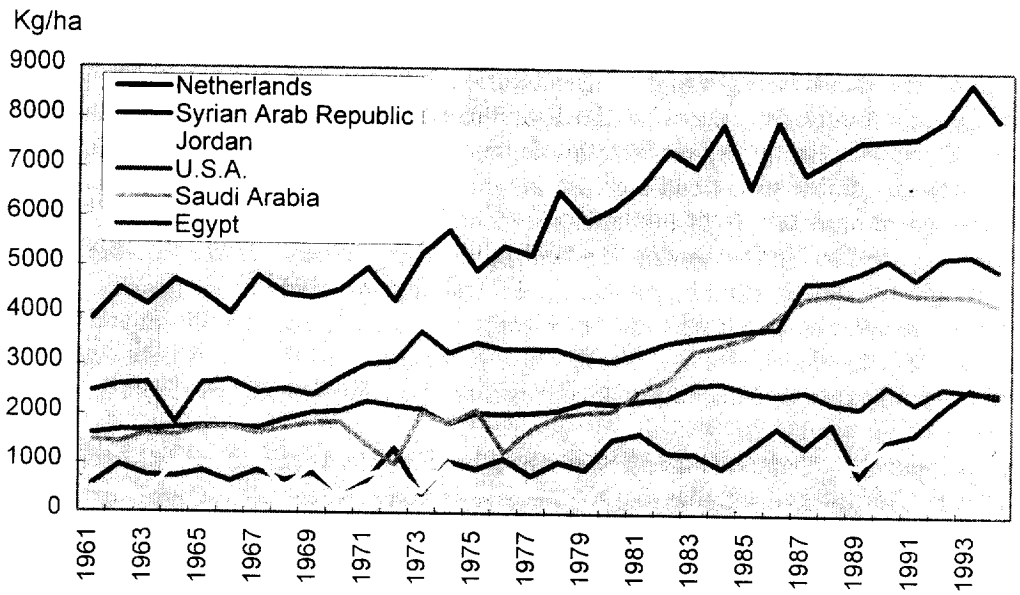
It may seem puzzling that the United States wheat productivity is considerably lower than that of Saudi Arabia and Egypt. However, it may be that the need to increase wheat productivity is less pressing in the United States as it is already one of the largest wheat-exporting countries, whereas countries in the Middle East are increasingly reliant on the import of agricultural commodities. Saudi Arabia is the major exception, having succeeded in becoming the sixth-ranking wheat exporter in the world. The downside of this development is a serious depletion of Saudi Arabia's underground aquifers. Moreover, the 1991/92 wheat harvest was estimated to have cost the Government of Saudi Arabia around US$ 480 per ton compared with world prices for wheat of US$ 100 per ton (22b).

Figure IV. Impact of successive technologies on agricultural productivity over time



In general, however, developing countries have a far greater need to increase agricultural production (i.e. productivity as well as cultivating more land) than developed countries. This is especially the case in the Middle East, where self-sufficiency ratios for main agricultural commodities have continued to decrease from an average of 95% in 1970 to 75% in 1989 (192).

Figure V. Productivity of wheat in selected ESCWA member countries, the Netherlands and the United States



Source: FAO, Agrostat (Rome, 1995).

In most industrialized countries, agricultural production has to comply with other demands besides expansion, including:

(a) Sustainability of production in the long term;

(b) Reduction of agricultural chemical pollutants;

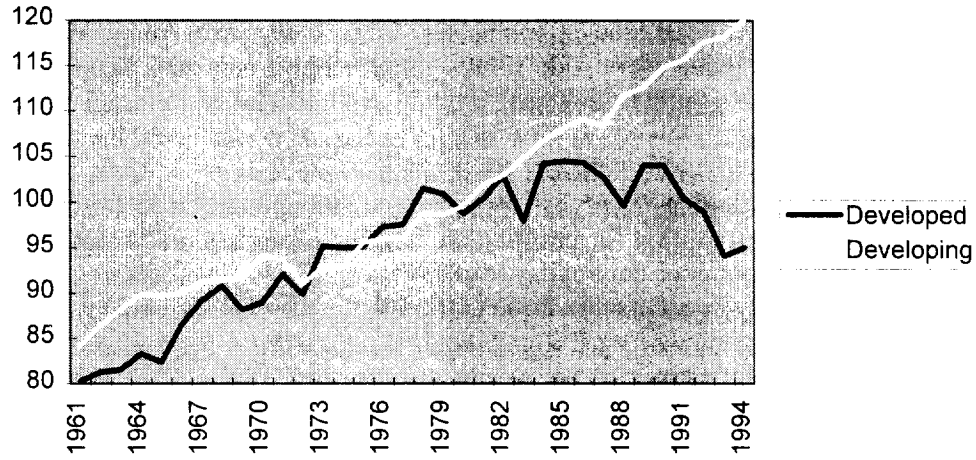
(c) Improvement of taste, shelf-life;

(d) Compliance with international standards to ensure access to international markets;

(e) Diversification of production.

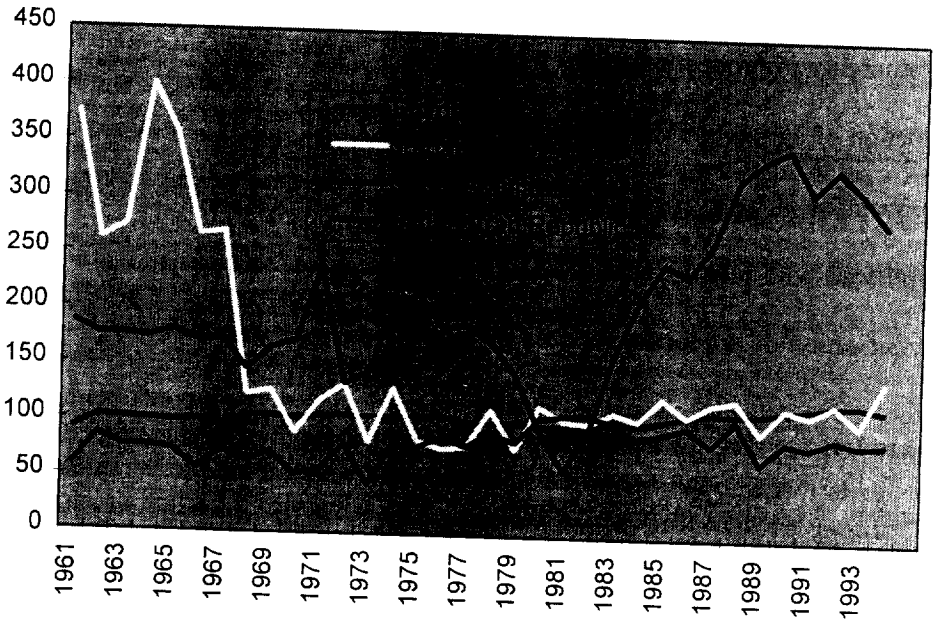
These factors have actually caused a decrease in agricultural production per capita in developed countries (see figure VI). In fact, the agricultural production per caput of developing countries is increasing exponentially. Both Egypt and Saudi Arabia are examples of this trend, but the Syrian Arab Republic shows only a gradual, widely fluctuating increase. Jordan has even witnessed a decline in agricultural production per caput (see figure VII).

Figure VI. Agricultural production index of developed and developing countries per capita



Source: FAO, *The State of Food and Agriculture 1996*, Print-out of Time Series for SOFA 96, FAOSTAT TS Software (Rome, 1996).

Figure VII. Agricultural production index of selected ESCWA member countries per capita



Source: FAO, *The State of Food and Agriculture 1996*, Print-out of Time Series for SOFA 96, FAOSTAT TS Software (Rome, 1996).

Of course, one cannot generalize such trends inasmuch as other, non-controllable factors such as international developments, politics, population growth and socio-economic developments play a decisive role in the final agricultural production figures. Nevertheless, countries in the Middle East have recorded impressive net growth in their agricultural production indexes. Since 1980, Saudi Arabia has been the largest grower in total agricultural production worldwide (26b). Surprisingly, number 2 is Jordan (26b). The dramatic rise in population figures caused the actual drop in self-sufficiency ratios of main agricultural commodities.

Usually, especially in developing countries, only the government-supported National Agricultural Research Systems (NARS) are responsible for ensuring an increase of agricultural production. The main activities of NARS consist of research on improving crop varieties and improved control of environmental factors. NARS have little influence over the other factors affecting the national agricultural production index. NARS cannot influence the allocation of agricultural land, the growth of the population, urbanization, available natural water resources, infrastructure, and pricing mechanisms. This underscores the need for more overall coordination through policies at the country level in order for NARS to become more effective.

International cooperation can be beneficial in establishing indigenous research capabilities over time. However, only if countries manage to be successful in establishing NARS by formulating and implementing policies will they become suitable partners for regional or international collaboration. According to the FAO:

> "The NARS are, and will continue to be the cornerstone of the global agricultural research system as they work to increase agricultural productivity and profitability in their own countries. ... The significantly increased numbers of well-trained scientists working in these NARS have been a very positive development of the past few decades. "(175)

There are strong indications that biotechnologies are on the verge of becoming the leading technological instruments of NARS for crop-breeding purposes in industrialized countries. The following section will shed more light on the extent to which biotechnologies may also be considered by NARS in developing countries as a useful tool for development and in what way the relevant technologies can be acquired. Because of the multisectoral nature of biotechnologies, more general aspects related to the commercialization of biotechnologies, such as patent issues, and technology transfer, will also be considered.

B. Biotechnology and development

Since the biological sciences were able to traverse barriers between species by using genetic engineering, some two decades ago, the biotechnologies have rapidly gained in popularity with scientists, investors and private companies. Simultaneously the general public started voicing concerns about the negative implications of biotechnology. As a consequence, expected benefits of genetic engineering were overstated, out of sincere enthusiasm or in order to attract more investment. This was naturally met by views from opposing camps with tales of uncontrollable transgenic micro-organisms causing irreparable damage to the environment.

Following the first wave of excitement, numerous committees, both governmental and non-governmental, were established specifically to address the consequences of biotechnology for society. At first, biotechnology was strictly taken as synonymous with genetic engineering. Soon, however, more rigorous definitions were formulated in which other bimolecular disciplines were included. The following definition is most appropriate in indicating the practicality of biotechnology (197):

> "Application of discoveries in biology to large-scale production of useful organisms and their products. Centres on development of enzyme technology in industry and medicine, and of gene manipulation, often in the service of plant and animal breeding."

Since biotechnology is primarily related to practical applications, commercial interest in the subject was soon generated. After the first dramatic scientific breakthroughs in the 1970s, it was between 1980 and 1984 that the majority of small specialized biotechnology firms were established in the United States (168b). A lack of any dividend payouts and large deficits have characterized the biotechnology industry from the very outset. Still, many small companies managed to capitalize on expectations that new breakthroughs will earn enormous profits. In addition, large venture-capital firms are able to sustain such investments over a longer time, and many multinational firms active in producing pesticides, pharmaceuticals and seed corporations view joint ventures, takeovers and disengagements as elements in long-term competitive strategies as well as a means of ensuring a viable position in tomorrow's state-of-the-art technologies. As a result, many giant agro chemical, pharmaceutical and food-processing transnationals began to dominate biotechnology research and the emerging biotechnology markets by the beginning of the 1990s (199c).

1. *Intellectual property rights*

Both private and public research institutes in the United States are becoming increasingly involved in the patenting of genes. Advocates of gene patenting claim that protection of intellectual property rights is a necessity for the biotechnology industry to grow, as this will supposedly provide commercial incentives to researchers to innovate and thus serve the public interest at large.

In fact the opposite appears to be true, as indicated in a recent study. Joly and de Looze (43a) claim that patents alone cannot induce a dynamic of technological differentation in the case of plant biotechnology. This is because most firms active in the field have similar technological profiles and many patents, at least partially, are overlapping. In genetic engineering of plants, there are many innovative technologies with the

same objective: a transgenetic plant. Therefore, patents in plant biotechnologies do not contribute to the coordination of actors' plans. This in turn removes the incentive to innovate.

So far, the developing countries are largely bystanders with regard to such developments. As with the Green Revolution, however, it is not so much a question of whether developing countries will be affected by biotechnologies, but rather when and in which sectors. Some effects are already becoming apparent when with regard to patenting.

Well-established companies are increasingly lobbying their Governments to push developing countries to adopt more stringent patent laws, even including the patenting of life forms and genes. The problem with living entities is that they violate such laws by their very existence: reproduction is part of the definition of life and takes place without human intervention.

Of course, biotechnology also offers the tools to limit reproduction with, for example, hybrid seeds, enabling companies to use patents strategically to keep a lead in the research race. This would allow more established companies to reap the benefits of many years of research and related investments. Often, however, most companies can only establish themselves by imitating or building on other technologies to allow further innovations to take place. Present developments in the international pharmaceutical sector may be taken as examples. In 1991, Hobbelink provided a sharp analysis on the historic development of patent pushing by industrialized countries:

> "But major OECD countries themselves started allowing for product patents on drugs only after their pharmaceutical industries had become firmly established: France in 1958, West Germany in 1968, and Switzerland in 1977. ... These figures might lead to the conclusion that the national pharmaceutical sector in many OECD countries was able to grow precisely because of the convenient absence of strong domestic patent protection for drugs. Only when export interests began to dominate did patent protection appear more desirable. The North, through its demands in GATT, is now seeking to deny the Third World that same route to development" (199d).

Another study indicated that there is no such thing as technical self-sufficiency of research institutions in areas in which advanced biotechnologies were developed into marketable products, such as monoclonal antibodies (46a). Research institutes could only develop such technology by building on significant pre-existing technological resources. This explains the close collaboration between academia and industry research on biotechnology in the United States, and puts the emphasis on accessing technologies from other countries.

2. *Technology transfer and development*

The acquisition of sophisticated biotechnologies generally presupposes the existence of adequate technological capabilities in a range of areas. This makes it difficult for developing countries to gain a foothold in this area. Surprisingly enough, the more advanced technology related to the production of monoclonal antibodies within the health sector (see chapter VI) was suggested by a recent study as a plausible area in which developing countries could explore the commercial opportunities of biotechnologies. Avramovic claims that:

> "one product line of biotechnology, monoclonal antibody-based diagnostics, is much easier to develop than other lines and is more viable within the resources available in developing countries for product development. The demand for these products is large and growing, capital requirements are relatively small, and product development times relatively short" (168c).

Indeed, entry into the field of diagnostics production would not only benefit the emergence of a biotechnology industry in developing countries but could render diagnostics cheaper and more generally accessible. However, the diagnostics industry is largely part of pharmaceutical companies and therefore restricted to the health sector. If technologies based on monoclonal antibodies are chosen as the basis for commercializing biotechnology in non-industrialized countries, this may result in the exclusion of other sectors, such as agriculture, to benefit from the same. It is precisely the multisectoral features of biotechnologies for which more universal policies should be developed at the country level.

There is an additional danger for developing countries in focusing on the more advanced biotechnologies. In most non-industrialized countries there is a lack of self-confidence concerning domestic technological capabilities. Foreign capital goods are often superior in performance and durability, feeding the incorrect presumption that domestic human and non-human resources are not sufficiently equipped to solve local problems. In turn this results almost automatically in an over-reliance on importing the entire set of required technologies, rather than developing proprietary technologies based on the blending of local capacities with the more well-established foreign technologies. Of course, this should not restrain developing countries from attempting the development of more advanced technologies as the basis for improving local R&D capacities. The importance of more refined modalities for international technology transfer was elegantly highlighted by Jason Zunsheng Yin, who wrote that indigenous technological capability factors are determinants of the economic and technological success of the transfer projects and that less developed countries should formulate a strategy for acquiring foreign technology to be consistent with their technological capability (171).

Examples of international biotechnology transfer, particularly in Egypt and Saudi Arabia, strengthen this observation. The most successful technology transfer project on genetic engineering of plants is being executed in Egypt. Since the National Agricultural Genetic Engineering Laboratory[1] (NAGEL) was established in 1989, the laboratory achieved a breakthrough in genetic engineering of potatoes by regenerating three commercial potato cultivars with a chimeric gene encoding the coat protein of potato X virus. Genetic engineering technology was established through technical assistance from UNDP and USAID. However, the genetic engineering techniques of NAGEL have, so far, not been made available to seed-distributing agencies or farmers. The indigenous technological capability of Egypt in this area is not yet ready for commercial use of the related advanced technology.

Another example of the relevance of indigenous technological capability to ensure the success of biotechnology transfer projects is in the area of date palm micropropagation in Saudi Arabia. This particular biotechnology is especially promising as traditional propagation through offshoots is expensive and time-consuming. In 1986, the Date Palm Research Centre of King Faisal University in Hofuf planted the first *in vitro* derived date palm, based on a somatic embryogenesis protocol. In international literature, somatic embryogenesis is seen as less promising than shoot culture as the former may lead to genetic variation of the propagated clones, making it difficult to predict whether the performance of such plants will be comparable to that of the mother plant. In subsequent years, three research institutes gradually developed the capacity to experiment and develop tissue culture protocols for date palm. The Date Palm Research Centre in Hofuf first established a more appropriate shoot culture protocol in 1995, and the National Agricultural Water Research Centre in Riyadh began large-scale production and distribution of tissue culture propagated date palms to farmers in 1992 (21,500 plantlets in 1995 (161)). The latter institute, however, is relying on a somatic embryogenesis protocol, with the consequent disadvantages. The actual first effective commercial technology transfer of date palm micropropagation materialized in September 1996, when a private Saudi Arabian company in Dammam (Saudi American Plant Development [SAPAD]) started the sale of plantlets

[1] NAGEL became the Agricultural Genetic Engineering Research Institute (AGERI) in 1992(66,74).

of 10 date palm varieties propagated by both somatic embryogenesis and shoot culture. The SAPAD proprietary micropropagation process was developed by ESCAGENETICS Corporation, an American public company.

Both examples indicate that it will take considerable time and effort to establish indigenous biotechnology capacity in ESCWA member countries. In addition, advanced agricultural biotechnologies are less likely as options for short-term technology transfer than well-established biotechnologies, such as the production of virus-free potato tubers by tissue culture.

There is not much reason to embark on genetic engineering of plants if the local technological capability (e.g. mastering of plant regeneration protocols, basic molecular biology techniques) is not sufficiently developed. It will not work to try to skip several phases of the technology development process by importing the entire set of required techniques as one complete technology package. Considerable time and planned efforts are required to first develop the more generic techniques which could ultimately culminate in the development of more advanced technologies such as genetic engineering. Only in those cases where considerable financial resources can be allocated for 20 or more years will it pay to invest directly in genetic engineering research of plants. In this regard, the following are noted:

(a) Rough estimates for 1995 suggest that the development of a genetically modified plant in the United States costs about US$ 10 million, of which US$ 1 million goes for regulatory expenses, and requires approximately six years to go from the laboratory to a commercial product (61b). By comparison, traditional breeding costs between US$ 2 million and US$ 2.5 million and requires a 6- to 10-year development period (61c);

(b) Although the first field trial with transgenic tobacco was conducted in 1986 (47a), genetic engineering of plants is still in the initial phases of technology development and adaptation. It will probably take another 20 years before the impact of genetic engineering on agricultural developments is fully felt;

(c) The United States biotechnology industry is still operating at a loss, despite the fact that the Government of the United States finances private and public biotechnology research amounting to more than US$ 4 billion annually (i.e. half of the total budget spent on biotechnology research) (17). The Government started programmes for the encouragement of new technologies (e.g. biotechnologies) in the last 25 years. In 1995, the American commercial biotechnology industry was operating at a loss of US$ 2 billion (8). Such losses were mainly incurred because of large R&D investments. By comparison, the Syrian Arab Republic's total R&D expenditure amounted to US$ 14 million in 1992 (177a). This implies that ICARDA, one of the international agricultural research centres, based in the Syrian Arab Republic, has a similar R&D budget (102a). Jordan, again, is in the same range with a public research budget of US$ 13.5 million. The total R&D expenditure of Egypt amounts to US$ 143.7 million, and that of Saudi Arabia amounted to US$ 131.2 million in 1992 (177a);

(d) It will become more difficult for developing countries to obtain advanced biotechnologies by direct ways of technology transfer (e.g. purchase, exchange programmes) because of the high initial investment requirements for advanced biotechnologies. Improved communications may not help developing countries as industrialized countries are diligently trying to protect their findings by pushing for more stringent patent laws.

3. *Biodiversity*

Despite the difficulties for non-industrialized countries to acquire and develop the more advanced biotechnologies, there is a pressing need for developing some capacity in this area. As plant genetic

resources form the backbone of any agrobiotechnology, developing countries are already experiencing exploitation by Western laboratories. Over time, most of the genetic variations of plants have developed in the tropical zones, including Western Asia. This has drawn the continuous and increasing attention of researchers from Western countries who can and probably will be able to obtain these genetic resources free of cost, although most of the vast genetic variations of crop species evolved from centuries of cross-breeding and cultivation practices by small-scale farmers.

Multinational, but also national, seed companies are encouraging farmers to switch to short-season high-yielding varieties whereby landraces are lost at a fast rate. It is here that the developing world as a whole stands to lose. National policies should be geared towards protection of these agricultural resources against further depletion. Within these policies, biotechnologies can be employed as a tool to protect against further losses of genetic resources and for improving plant breeding programmes.

There are several stages through which capacity-building in technologies for the conservation of plant genetic diversity can be achieved. First of all, an inventory should be made of what is actually left of the original biodiversity of important crop species in the region. The genetic variations within a given crop species are of interest in this respect. When it comes to cultivated crops, developing economies have an advantage in that some agricultural producers are marginalized, which means they have to rely on their own seed resources. It is in such contexts that a virtual wealth of genetic diversity within crop species can be found. Most of the time, such land races, or local varieties are characterized by low yields or other undesirable characteristics as defined by modern economies. However, when it comes to adaptation to harsh climatical conditions or resistance to a variety of pests and diseases, such local varieties might prove to be invaluable for crop breeding programmes in the long run. Moreover, what is currently desirable in terms of pest- and disease- resistance, tolerance to climate stress, preferred characteristics for consumption and by-products may well change in the years to come.

Therefore, crop-breeding programmes of individual countries in the ESCWA region can benefit considerably by identifying and preserving these land races. As a first step, the natural habitat of crop species can be identified and protected. To avoid a conflict of interests with local users of such natural habitats, policy can incorporate interactive modes with local communities. This would allow for the protection of local users' interests, as well as the protection of the natural habitat. Moreover, the indigenous knowledge of local communities can then be explored to identify and characterize plant species. Integrated teams of anthropologists and taxonomists are to play a key role in this interaction with local communities. Identified land races or plant species of interest can be stored in gene banks in various modes (plant and seed collections, tissue collections, cell-line collections and genome collections). For the interest of intellectual property rights concerning conservation of genetic biodiversity, special attention can be awarded to crop species originating in the region. To benefit from experiences at the regional level, national gene banks can establish links with other gene banks in the region to exchange material and information.

As a second step to have crop-breeding programmes benefit from genetic biodiversity resources, national research institutes can establish technologies to identify plant species more specifically. In addition to established procedures of determining different genotypes by phenotypic traits or allelic frequencies, efforts should be directed to genetically characterizing genotypes, using genetic markers (RFLP or RAPD) or immunological diagnostic techniques. This would speed up identification procedures considerably as well as avoid duplication of the laborious work in growing plants to full maturity. If such technologies could be established, it would help both the preservation of biodiversity and crop breeding. Arab countries would be able to establish a proprietary position concerning identified genotypes to be used in crop breeding programmes. Finally, Arab countries would be able to regulate access to these identified crop varieties, to some extent. It must be emphasized, however, that non-industrialized countries like the member States of the ESCWA region are in no position to compete with industrialized countries at a technological level and

will be dependent on technology transfer to fill the current gap. However, there is ample incentive to explore international modes of technology transfer for characterizing genotypes and developing new crop varieties.

Paradoxically, the same biotechnologies can be used for *in vitro* protection of bio-resources and for the breeding programmes that have created the new superior plant lines replacing the land races. It is precisely in consideration of such issues that biotechnology policies are needed.

C. Policy recommendations for organizing biotechnology research in selected ESCWA member countries

An analysis of the need for biotechnology solutions and commercial prospects cannot be framed in general terms. Each of the ESCWA member countries surveyed (Egypt, Jordan, Saudi Arabia and the Syrian Arab Republic) is very distinct in social and economical parameters. Even more so, at the micro-level of individual farmers, agricultural and socio-economic conditions are increasingly diverse. The general lack of any modality of organization of farmers and other interest groups has strengthened this diversification and, for this reason, a thorough analysis of market demands and farmers' interests at the grass-roots level is required, before any attempt can be made to implement biotechnology policies.

Good governance is increasingly being recognized as an essential condition for sustainable development at the country level (169). Concerning advanced biotechnology research, the need for sustainability in securing resources for a longer time period is apparent. In order to analyse appropriate mechanisms for good governance, tools available to policy makers must be identified. Three immediate objectives for policy makers can be of assistance in demonstrating how policy formulation and implementation can improve the effectiveness of national research:

(a) First, national policies should strive to ensure growth in research capacity over longer time periods. Research is a long-term endeavour and needs a continuous supply of funds for obvious reasons.

(b) Secondly, policy makers may wish to boost the motivation of research personnel so as to increase performance. In addition to financial incentives, research staff can be motivated by providing them with more authority to access resources. In other words, the aspirations of research staff to have control over their personal careers "is fundamentally about people: releasing and harnessing their productive potential and satisfying their human needs and desires" (198). Mechanisms that allow for more competition among researchers or research institutions constitute another instrument available to policy makers to increase the motivation of personnel.

(c) Thirdly, policies can be used to improve the efficiency of management. A reduction in management layers will result in less distortion of communication in the process of information exchange between the actors involved. This simple principle may be applied by reorganizing agricultural research as follows:

(i) Ensure domestic technology transfer between researchers, technology developers, technology producers, technology distributors, technology users and consumers;

(ii) Ensure feedback mechanisms from technology users (e.g. farmers) and consumers (domestic and foreign markets) to allow for mechanisms to increase the quality of the technology;

(iii) Ensure more complementarity of the actors to allow for safety valves in the National Agricultural Research System.

Coordination is another instrument available to policy makers. Egypt, Jordan and the Syrian Arab Republic currently lack national agricultural research coordinating mechanisms (174). Saudi Arabia has already established a National Council for Agricultural Research with authoritative coordination responsibilities. In the case of the Syrian Arab Republic, no fewer than three ministries (the Ministry of Agriculture and Agrarian Reform, the Ministry of Economy and External Trade and the Ministry of Irrigation) are involved in the planning of public agricultural research (192a).

Most emphasis is on accessing and increasing the cross-fertilization of local technology resources. This could well form the groundwork for accessing and improving foreign technologies to better contribute to enriching local technological capabilities. Additionally, greater emphasis will have to be placed on the role to be played by national human resources since only they will be able to sustain research efforts over longer time-frames. As already indicated above, human resources in the surveyed countries are not lacking in expertise or numbers, especially in the case of Egypt. Of course, there is nothing wrong with utilizing foreign technologies, but core personnel in the technology transfer chain (e.g. researchers, entrepreneurs, retailers, farmer-representatives, public administrators) should be mainly local to allow for the sustainable maintenance, development and adaptation of technological goods to domestic settings.

The final sections of this study will attempt to illuminate for policy makers some specific areas of interest where biotechnology can improve or enable production or services. Such recommendations are made on the basis of previous chapters. Biotechnology is foremost regarded as a tool and not as a prestigious development target. All sectors to which biotechnology may contribute are included. Policy recommendations are targeted at the country level. To induce cooperation among national entities at the regional level, specific recommendations are included to cater for more coordination between the ESCWA member countries.

1. *Egypt*

Egypt's commercial activities in biotechnology can be found in virtually all relevant sectors: agriculture, fermentation industry and medicine. Private enterprise initiatives in biotechnology, however, are confined to two companies active in plant tissue culture. Industries using biotechnology have not been privatized so far. Some public research institutes have developed commercial production of goods or services with varying success. AGERI confirmed the sale of ELISA (Enzyme Linked Immunosorbent Assay) kits to one of the privately operating companies in tissue culture, worth approximately US$ 15,000. This amply suffices to pay the salary of two senior research workers in Egypt. The plant cell and tissue culture department of the NRC reported the sale of micropropagated plantlets, worth approximately US$ 3,000. Although far less than the amount raised by AGERI, this amount constitutes 20% of the yearly research budget of this department of NRC.

Illustrative of the influence of external funding on developments in Egypt is the comparative size of foreign assistance. Egypt was the main recipient of ODA (Official Development Assistance) both among the ESCWA member countries and worldwide in 1992-1993 (see table 29). It is clear that the way in which funding of biotechnology institutes takes place in Egypt has a considerable effect on biotechnology transfer and development.

One of the negative side-effects of external funding is the uneven distribution of financial resources among research institutions working on biotechnology in Egypt. Clearly AGERI is the most established institute working on genetic engineering of plants, thanks, in part, to the considerable financial and technical support provided by USAID. Since AGERI has become a centre of excellence in Egypt, researchers from many other Egyptian institutes are attracted to AGERI. Naturally this makes it more difficult for other institutions to also build a strong research programme. The difference in quality of research institutions is

illustrated by the gap between AGERI and the Genetic Engineering Centre of Cairo University. The Genetic Engineering Centre ranks second to AGERI in terms of human resources, equipment and research achievements. Since NAGEL, the predecessor of AGERI was established in 1989, there is a gap of 7 years between AGERI and the Genetic Engineering Centre, which was only established in 1995.

TABLE 29. STATUS OF ODA RECEIVED BY EGYPT AS COMPARED WITH OTHER ESCWA MEMBER COUNTRIES AND WORLDWIDE

A Country	$ million	B Country	Percentage total ODA	C Country	Percentage total ODA
Egypt	2,256	Egypt	5.5	Israel	13.0
Yemen	336	Indonesia	3.9	Egypt	10.5
Jordan	317	China	3.5	El Salvador	4.3
Iraq	170	Israel	2.7	Somalia	3.2
Syria	168	Philippines	2.7	Philippines	2.2

Notes: A: Main recipients of ODA in the ESCWA region in 1992-1993 (6a). B: Main recipients of total DAC (Development Assistance Committee) members aid as percentage of total ODA gross disbursements in 1992-1993. Total ODA 1992-1993: US$ 68,821 million (6b). C: Main recipients of United States aid as percentage of total ODA gross disbursements in 1992-1993. United States total ODA 1992-1993: US$ 12,401 million (6c). (Numbered references correspond to references for this study.)

These negative aspects are less apparent with developments in plant cell and tissue culture. Plant cell and tissue culture techniques have been mastered by the majority of research institutions working on plant biotechnologies. There is extensive collaboration and mutual benefits gained by research institutes and private companies active in this field. This might well be because most Egyptian academic institutions have been involved in PCTC over the last decade, providing a broader base and therefore allowing for horizontal institutional technology transfer in Egypt.

All Egyptian institutes visited were of the opinion that the most restricting factors for biotechnology transfer and development are the lack of sufficiently trained manpower and lack of access to chemicals and laboratory supplies. The training level of manpower, however, does not seem an impediment as the majority of research workers in Egypt have received training overseas (13 out of 14 researchers interviewed), confirming the same observation made by the ASRT. More relevant is the lack of access to equipment and chemicals. To import chemicals and laboratory supplies into Egypt requires a government procedure of two weeks (66). As biotechnology requires a lot of short-life chemicals such as enzymes and radio-isotopes, quality and planning of research activities is severely hampered. Concerning equipment, there are currently no technically capable agents in Egypt to ensure sufficient spare-parts, maintenance and guidelines for operation (78).

At present the Egyptian Government has not set any priorities as to which sectors are to benefit from biotechnology. ASRT suggests that the primary focus should be on agriculture. A UNIDO consultant remarked that "it is felt that a very wide gap exists between the results of the current international research (in agriculture) and the reality in Egypt." Therefore " the research pertaining ... (to) agriculture is of top priority" (68c).

From the findings of this study, it can be seen that agricultural research in biotechnology receives far more support than other sectors such as industrial fermentation and medicine. The latter two sectors promise

a more direct return on investments in advanced biotechnologies. The in-house development and production of enzymes using bioreactor technology could directly feed into more commercial gains in the detergent—and food—industry, and for the production of clinical diagnostic enzyme kits. Investments for expanding molecular biology techniques for cancer diagnosis in medicine would directly decrease overall health bills. Egypt should award greater priority to those sectors, when considering biotechnology.

The following observations may be made as a result of the preliminary ESCWA survey of biotechnology activities (in which Egypt is relatively more advanced). There is primarily the need to formulate a clear strategy for biotechnology research. Despite the high quality of available human resources, the research infrastructure and funding patterns remain seriously in need of coordination. Greater benefits can be obtained if research institutes reorganize and adopt market-oriented policies and organizational structures to stimulate income generation for operation and expansion. Existing institutions should also consider establishing networking and other arrangements to ensure an effective division of labour among themselves. This should create a more competitive scientific environment which would enhance the research potential in general. External assistance may then be used to more evenly support research groups over a longer time span. Finally, institutions active in biotechnology research should implement modes of planning and operation that directly link their research with commercial initiatives.

2. *Jordan*

This section will first describe general positive and negative observations affecting the development and implementation of biotechnologies in Jordan. An attempt will be made to indicate the impact of biotechnologies on the national economy. Thereafter, important biotechnology cases in Jordan will be highlighted, as well as impediments to and solutions for further development. The section will conclude with policy recommendations on improving access to the benefits of biotechnologies for selected sectors.

The emergence of a science and technology policy, as formulated by the HCST and applicable to the 1993-1997 Jordanian Development Plan, implied a major positive change in comparison with previous development plans. According to Zahlan (15a), the science and technology section in Jordan's 1986-1990 Development Plan "lacked two essential ingredients to make it useful: it had no science policy content; and it did not reflect the construction or promotion of a science and technology system".

Currently, in addition to developing a science policy, the HCST is working on the means to execute those policies. The first achievement is the establishment of a scientific research fund, with the support of the Ministry of Planning. Depending on further Government agreements, the HCST may be involved in the legislation process for the purpose of providing a suitable scientific climate and furthering the implementation of the strategies adopted (191a).

(a) *Positive and negative observations on biotechnology developments in Jordan*

According to a classification used by UNESCO for categorizing a country's developmental state in biotechnologies (5j), Jordan would belong to the lowest category of developing countries, that is, countries with an interest in but no direct involvement in modern biotechnologies.

This categorization of a small country like Jordan, with few natural resources, and surrounded by powerful neighbours is, however, not sufficiently specific to describe the status of biotechnologies in Jordan. In addition, it is questionable whether involvement in modern biotechnologies would directly benefit Jordan. It may be a better strategic choice to first establish sustainable technical capacities in more accessible technologies. Biotechnology developments in Jordan are not stagnant, but rather dynamic and allow for flexibility, as noted by the following observations:

(a) Biotechnologies have been included in Jordan's policy for Science and Technology (e.g. as a main component of a package of technologies (see box 3).

(b) Biotechnologies are integrated with other disciplines to allow for a problem-solving-oriented approach rather than a technology supply-push.

(c) Jordan's main activities in biotechnology are in areas (e.g., production of virus-free potatoes, production of veterinary vaccines, integrated pest management) where a return on investments, or anticipated benefits for target groups is most likely to occur.

(d) Incentives, in the form of small research grants, are provided to researchers in many different biotechnology areas to improve research capacities and to make research results more suited to interested parties (private and public).

(e) Basic molecular biology research (e.g., the cutting and joining of DNA, PCR experiments) are carried out by most public research institutes. This is a good start for building the necessary capacity for genetic engineering.

Negative observations about biotechnology developments in Jordan are more general in nature and also apply to other technologies. The development and implementation of technologies in Jordan is hampered by the following restrictions:

(a) A sustainable level of public research funding is not available. The yearly research budgets of institutions tend to decrease directly after their establishment to reach a level at which only salaries for staff members are available.

(b) As with most countries in Western Asia, the management of human resources does not allow for many incentives or rewards to improve scientific competence.

(c) A majority of senior research personnel in Jordan have received training in industrialized countries and are familiar with better research support (e.g. equipment, salaries, communication, travel allowance) than that available within the research framework in Jordan.

(d) Technology transfer and development is viewed as an activity for promoting local research capacities by accessing outside sources. There is little emphasis on technology transfer in the sense of means through which local basic and/or applied laboratory research and innovations are brought about for the public use and benefit.

(e) The lack of national coordination of biotechnology activities has led to duplication of research.

An attempt will be made to analyse the influence of the negative and positive observations on the contribution of biotechnology research and development to the Jordanian economy.

(b) *The impact of biotechnologies on the Jordanian economy*

Investments in a country's research and development (R&D) capacities are generally seen as an essential but uncertain undertaking to ensure a sustained level of competition with other economies, in order to produce goods and services. Often the proportion of R&D to the GDP or GNP of a country is used to determine whether investments in R&D should be increased. In the case of Jordan, R&D expenditure as a percentage of GDP amounted to 0.28% in 1992 (177b). According to UNESCO and other sources, this ratio

should be at least 1%. However, although Jordan's proportional spendings on R&D are far less than industrialized countries, they are well above the R&D expenditure of other countries in the ESCWA region. The drawback of this indicator is that it does not measure the contribution of local scientific and technological capacities to the production of national goods and services.

A better indicator may be to compare import figures of capital goods and services to export figures of the same, over time. This would indicate to what extent a country is becoming increasingly reliant on foreign goods and services, and thus on foreign technology which is necessary to produce these goods and services. In the case of Jordan, there is a negative balance on goods and services from 1988 to 1993 (see table 30), indicating that Jordan is still over-reliant on foreign technologies.

TABLE 30. EXPORT AND IMPORT OF CAPITAL GOODS AND SERVICES, JORDAN (JORDANIAN DINARS)

Year	Population in millions	Export of capital goods and services per capita	Import of goods and services per capita
1988	3.001	304	471
1990	3.453	452	690
1991	3.888	308	608
1993	4.152	472	756

Sources: The Economist Intelligence Unit, *Country Profile Jordan 1992/93* and *Country Profile Jordan 1994/95* (London).

Notes: The decrease in both export and import figures in 1991 was due to the Gulf war.

The weakness of the export to import ratio of capital goods and services as an indicator of the impact of biotechnology R&D on improving economic output is clearly illustrated in the case of the Jordanian pharmaceutical industry. Although this sector accounted for more than 10% of total export figures in 1994, imports of foreign capital biotechnology goods of up to US$ 29.6 million (i.e. 29% of exported value of pharmaceuticals in that same year) were required for the Jordanian production of medicines (9a).

At first glance, it appears that the short time frame for developing biotechnologies does not allow for any impact on boosting production in related sectors of the national economy of Jordan. Most biotechnologies were only introduced at research centres at the end of the 1980s. The majority of activities in biotechnology can, therefore, only be found in research. Two biotechnologies, however, have resulted in the production of goods and services. These technologies are the virus-free micropropagation of potato and the production of veterinary vaccines.

The implementation of biotechnologies supporting the production of goods and services only takes place in the agricultural and livestock sector, specifically the propagation of potatoes and the production of veterinary vaccines. No biotechnology research or implementation activities of importance are being carried out to support plant breeding of Jordan's main crops, which include wheat, barley, tomatoes and other vegetables, olives, grapes, citrus fruit, melons and bananas. Despite this lack of contribution by biotechnologies to agriculture, the growth of Jordan's agricultural production has been the second largest in

the world.[2/] The performance of Jordan's agriculture in terms of yield per hectare also shows a positive trend, except for wheat for which total production and yields per hectare decreased between 1992 and 1994 (26c). It seems that an increase in domestic resource cost removed incentives from farmers to increase production and productivity in the case of wheat. Needless to say, Jordan is still very much in need of boosting agricultural production as the country cannot meet its own food requirements. As in most countries in the region, the main restriction on agricultural production in Jordan is the lack of sufficient irrigation water, a problem for which biotechnologies offer no immediate solutions to date.

(c) *Potato biotechnology*

Biotechnology for crop improvement only reached the implementation stage for potatoes. In terms of total area harvested, the potato is an insignificant crop for Jordan (see table 31). However, Jordan almost managed to reach self-sufficiency in potatoes over the last decade, indicating a continuous and growing demand for superior seed-tubers.

TABLE 31. POTATO PRODUCTION, EXPORTS AND IMPORTS IN JORDAN

POTATO	1980	1992	1993	1994
Area harvested (1,000 HA)	1	2	5	3F
Production (1,000 MT)	9	49	118	70F
Exports (1,000 MT)	4.2	2.6	2.8	4.6
Exports (1,000 US$)	1,075	659	730	1,633
Imports (1,000 MT)	31.6	9.6	9.5	13.8
Imports (1,000 US$)	9,190	4,493	3,967	5,212
Self-sufficiency ratio	25%	88%	95%	88%

Sources: FAO, *FAO Production Yearbook 1994* (Rome), pp. 89-90; *FAO Yearbook, Commerce 1994*, p. 114; and *FAO Trade Yearbook 1981*, p. 137.

Notes: F = FAO estimate

Self-sufficiency is calculated by: $\frac{\text{production}}{\text{(production + imports - exports)}} \times 100$

HA = hectares; MT = Million tons.

Jordan's potato productivity in terms of yield per hectare is the highest among the ESCWA member countries, reaching 24 tons[3/] per hectare in 1993 (see table 32). The limited cultivation area and the comparatively high yield limit, limits the potential contribution of virus-free potato seed tubers to increasing potato production in Jordan. There is, however, great incentive for private Jordanian companies to explore the production of high quality potato seed tubers as 75% of Jordan's requirements are currently

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[2/] The agricultural production index of Jordan reached 243.89 in 1994, from a base of 100 in 1979-1981 (26b).

[3/] The world's highest potato yields are recorded in Belgium-Luxembourg, amounting to 49 tons in 1993 (26d).

imported (88). The investment margin available for producing virus-free potatoes allows four to five small[4/] companies to operate on the Jordanian market, unless market access could be obtained to other countries in the region. An added benefit for exploring the production of virus-free potatoes in Jordan may well be a lower unit price of seed tubers, thus benefiting farmers. Jordan could also save up to US$ 3.4 million in foreign currency if imports could be replaced by local production of seed tubers (126a).

TABLE 32. YIELD OF POTATOES IN ESCWA MEMBERS

Country/area	Potato yield in kilograms/hectare			
	1979-1981	1992	1993	1994
Bahrain	25,000	18,800	16,286	15,625
Egypt	17,399	20,907	21,250	21,333
Iraq	18,464	17,917	17,474	17,447
Jordan	16,866	23,505	24,013	23,333
Kuwait	16,934	17,500	20,000	19,659
Lebanon	16,947	21,035	20,438	20,683
Oman	13,663	22,979	22,917	23,024
Occupied territories	18,333	44,091	22,273	24,364
Qatar	13,367	9,773	10,000	10,909
Saudi Arabia	9,931	17,448	17,871	17,979
Syrian Arab Republic	15,302	16,906	17,687	17,631
United Arab Emirates	14,558	19,000	20,486	20,374
Yemen	11,992	13,569	14,056	13,698
WORLD	14,168	15,033	15,931	14,591
Highest yields	39,246	41,607	49,404	39,942
	Belgium-Luxembourg			
Lowest yields	7,273	4,000	4,000	4,000
	Angola			

Source: *FAO, FAO Production Yearbook 1994* (Rome), pp. 89-90.

The great number of public and private initiatives in Jordan for producing virus-free potatoes promises severe competition, which may be of interest to farmers as the quality of potato seed will increase whereas price levels may remain low owing to anticipated competition. Concerning investment in R&D, this trend

[4/] "Small company": a workforce of 20 persons or less.

reflects a waste of financial and human resources, as some plant tissue culture initiatives are bound to fail in the competition. Investments in biotechnology research can better be geared towards Jordan's main crops. Both public research and private research serve the country's interests and help to obtain access to bigger markets. If Jordan wants to develop more proprietary technological capacities in agrobiotechnology, public research could be directed towards crop improvement of almonds, pistachios and olives. These crops have their origins in the Mediterranean region, giving countries like Jordan an advantage by its genetic biodiversity of these species.

(d) *The production of veterinary vaccines*

In the area of livestock production, the joint initiative of the Ministry of Agriculture and GTZ (Gesellschaft für Technische Zusammenarbeit: German technical aid organization) in producing veterinary vaccines appears to better serve the country's interests. The country's sheep flock doubled in numbers between 1987 and 1994, to a total of 2.1 million (26e), increasing demand for veterinary vaccines to maintain animal production figures. The major success story has been poultry and egg production. The Kingdom is now self-sufficient in both, though with occasional interruptions in the supply of broilers owing to disease and insufficient slaughterhouse and refrigeration facilities (59a). The Jordan Centre for Veterinary Vaccines has filled part of this gap by directly distributing vaccines to livestock owners. The economic sustainability of the Centre, however, appears weak as production costs are higher than revenues from sales, and export of vaccines to other countries in the region is difficult, depending on trade agreements. In addition the Jordanian private sector is not interested in investing in the enterprise. Strict Government regulations would hamper the flexibility of the Centre's operation to seek new markets. This may result in lack of financial capital to invest in research and development or even to ensure quality of production in the mid-term.

(e) *Policy recommendations for national coordination of biotechnology activities in Jordan*

Jordan, so far, is the only country in the ESCWA region in which national policies have incorporated biotechnologies. HCST has made a start with the implementation of such policies by actively supporting and monitoring initiatives and research in biotechnologies. Private companies and some research workers complain that this support is not sufficient. Companies have trouble with importing certain chemicals, plant material and biotechnology products which do not receive special treatment in customs, thereby causing the destruction of many temperature-sensitive materials. Clear guidelines or incentives for experienced biotechnology research workers at universities to initiate or collaborate with private biotechnology enterprises are lacking.

Government officials responsible for biotechnology policy formulation and implementation have hinted at a lack of interest from the scientific community as the reason a newsletter on biotechnology initiated by HCST failed after three years (94). The third group of actors, consisting of Jordanian private investors, is mainly interested in short-term profits on their investments and are unwilling to make the large investments needed for the development of technology and building infrastructure facilities (94, 60b). Obviously, poor communications between national research and the private sector are one of the main obstacles to finding new applications in biotechnology (60a). The difficulty in linking demand in industry and agriculture with research activities seems to be due to a lack of confidence of industrial producers in local research and development. Therefore, industrial producers would rather import technology than develop proprietary technology. The import of foreign biotechnologies is even advocated by agriculture policy makers in Jordan. According to Arabian (62a): "Limited domestic resources do not allow us to consider technology generation as a rewarding option for the achievement of national goals [in Jordan]". This observation is, however, strongly opposed by international research findings, which indicate that indigenous technological capability factors are key determinants of the economic and technological success of technology transfer projects (171).

In other words, local technology generation or adaptation of imported technologies is the only rewarding option to choose for establishing an efficient science and technology base to support the national economy.

It is obvious that the development and commercialization of biotechnologies are still in their infancy in Jordan. Research in biotechnologies will need more long-term investments to boost capacity and thereby the confidence of investors. This is clearly a vicious circle; without investments the research bodies will not build more capacity to obtain more investments. The intermediatory role of the Government of Jordan is required to bring both sides (research bodies and investors) together and to provide incentives for investments in biotechnologies, as well as supply legislative support for problems (such as those at customs) to improve the prospective returns on investments.

Based on the above, the following suggestions are made for formulating policies to increase the efficiency of biotechnology transfer and development in Jordan. Jordan would benefit from more national coordination on biotechnology to achieve the following:

(a) Unite the interests of public research and private companies;

(b) Involve all interested parties in identifying national demand issues and interests of biotechnology suppliers and beneficiaries;

(c) Avoid duplication of public research efforts (as is the case with the production of virus-free potato seed tubers);

(d) Assure a national regulatory framework for the import and export of biotechnology-related raw materials and products;

(e) Reduce Government legislation that negatively influences biotechnology transfer and development.

Since biotechnologies have only been recently established, Jordan would benefit from more flexibility in public research spending, allowing for different mechanisms and levels of Government support and involvement in R&D activities, depending on the prospective economic benefits of biotechnologies. Mechanisms for linking technology demand with R&D in biotechnology may include:

(a) In the case of generic biotechnologies (e.g. micropropagation of selected crops) with low investment risk characteristics, private companies should be allowed to absorb research groups totally with continued financial Government support. Government support could continue until the research department has been absorbed in the production line of the private company, or has become self-reliant in terms of financial revenues.

(b) Alternatively to (a), the Government can allocate a special research budget for sponsoring R&D activities in generic biotechnologies up to a previously agreed amount. A precondition could be that interested organizations (e.g. research bodies, private companies) should all collaborate and that the technology is vital to the organizations.

(c) Special Government grants could be awarded to research initiatives that are both innovative and related to products for the export market; HCST already harbours a special research grant fund for innovative research; HCST may thus include extra conditions related to the relevancy of biotechnologies for the export market.

(d) In the case of medical biotechnologies, the pharmaceutical industry and hospitals could unite to establish a joint medical research fund.

(e) In the case of agricultural biotechnologies, target groups (i.e. agricultural producers) are to be united to establish private research funds.

To allow for the involvement of all relevant actors in the process of national coordination of biotechnology transfer and development activities, biotechnology focal points are to be established with the interested public and private sectors. In Jordan the following sectors have an interest in biotechnologies:

(a) Private agricultural supply and marketing companies.

(b) The private pharmaceutical sector. In March 1997 a seminar on biotechnologies for pharmaceutical manufacturers was scheduled to be organized in Jordan by the Arab Union of the Manufacturers of Pharmaceutical and Medical Appliances (AUPAM). Jordanian private pharmaceutical companies have committed themselves to financing part of the seminar.

(c) The agricultural research sector represented by the National Centre for Agricultural Research and Technology Transfer.

(d) The food-producing industry.

Encouragement of promising researchers could be achieved through incubator projects. The human resources of Jordan in science and technology are noteworthy in the ESCWA region. Scientists who have promising biotechnology research agendas may be supplied with added incentives in the form of research grants, and business and commercial training.

3. *Saudi Arabia*

The economy of Saudi Arabia is dominated by petroleum, of which the country has been the largest producer within OPEC for many years, accounting for 13.5% of global oil output in 1992(201b). Obviously, because of vast financial revenues from oil exports, Saudi Arabia has witnessed drastic changes in the structure of its society and economy over the century. To better direct the rapid modernization in Saudi Arabia, the national economy has been guided by a series of five-year plans since 1970.

A major strategic shift in internal policies took place with the fifth five-year plan (1990-1995) whereby the role of the private sector received increasing attention. Despite its intentions, the Saudi Arabian Government seemed to back away from privatizing major industries (22a). Additional elements of the five-year plan are job opportunities and training for the Saudi Arabian labour force; import substitution and the promotion of exports; and the diversification of economic activities into non-oil areas.

One of the more important strategic non-oil sectors in Saudi Arabia is agriculture. Agriculture serves to diversify the economy, creates job opportunities, ensures self-reliance in major food commodities (e.g. cereals, eggs), and, increasingly in the last few years, largely contributes to non-oil exports (40% in 1988) (201a). Over the Past five years, agriculture contributed approximately 9% to the GDP (22c). About 35% of the total work force was active in agriculture in 1994 (202a)

Agriculture opportunities in Saudi Arabia have their limitations as only about 2% of the land area is suitable for cultivation. In addition, available water resources curtail further expansion of this sector as an estimated 85%-90% of the country's water reserves are devoted to agriculture use (22a). The main threat

to the sustainability of Saudi Arabia's agriculture is the heavy strain on its non-renewable fossil aquifers from which farmers draw most of their water supply. The aquifers are being increasingly depleted thanks in part to advanced drilling technology made available through the oil industry.

Nevertheless the Government is continuously trying to increase access of the country's water supply through accessing more aquifers and constructing dams and irrigation and drainage networks. The aim of this programme is to raise agricultural production to the level of near self-sufficiency in all food commodities (201a). As noted above, Saudi Arabia has achieved the highest agricultural production growth in the world over the past 15 years. This emphasis on increasing production at all costs has led to cases of extreme water consumption. For instance, it has been calculated that dairy farming in Saudi Arabia uses 1,500 litres of water per litre of milk production (201b).

In order to maintain stability in the agriculture sector, the Government has encouraged production by several support mechanisms. In addition to subsidies on key commodities (e.g. wheat, barley), interest-free loans are available to farmers. Chemical fertilizers, farm machinery, irrigation pumps and imported rearing stock can be purchased at reduced prices (201b).

It may be clear that most support for farmers to improve and increase agricultural production is through financial incentives. Less emphasis is put on research and development. Saudi Arabia spends 0.11% of its GDP on R&D, which is the lowest figure among the ESCWA member States (177b). Food and agriculture research received a comparatively high proportion of 23% of KACST project funds in the period 1978-1992 (totalling SRls 265.90 million (148a)).

In sharp contrast with Egypt, Saudi Arabia does not have to rely on development aid to ensure access to foreign R&D capacity and technology. Instead, Saudi Arabia is one of the major aid donors in the world, with total disbursements averaging 2.9% of its GNP during the period 1980 to 1993 (203b). For Egypt, a positive side-effect of receiving aid money is the technical assistance which goes along with development aid.

Therefore, Saudi Arabia is filling its demand for foreign technical assistance by directly hiring experts from around the world. Many of the highly trained workers in Saudi Arabia are from the central-south Asian sub continent and the Arab world. Non-nationals from the Arab world account for 40% of the 6.26 million foreign workers in Saudi Arabia (203a). Many highly trained scientists from other Arab countries have been employed in Saudi Arabia, adding to a depletion of human resources in their home countries. For instance, the most reputable Arab scientist active in date palm micropropagation is Syrian and is currently working in Saudi Arabia, where he successfully developed an organogenesis protocol for date palm. However, attempts by the Syrian General Organisation of Seed Multiplication to develop date palm micropropagation protocols were not successful.

Of course, there are also some obvious benefits to the employment of Arabs of other nationalities in Saudi Arabia. Saudi Arabia is in a position to speed up its R&D activities, and nationals from other countries will provide important feedback to their home countries once they return, as they have been operating in a scientific system which provides more incentives to perform well.

Agricultural research in Saudi Arabia to improve crop performance is largely confined to public research institutes and is coordinated through the Ministry of Agriculture and the King Abdulaziz City for Science and Technology, as described above. The main opportunities for agrobiotechnology research to improve the production of selected agricultural commodities are to be found with the date palm and potatoes.

(a) *Potato biotechnology*

As can be seen in table 33, Saudi Arabia has witnessed an impressive increase in potato yields as measured in kg per hectare, since 1980. However, the country's yields are below half of the world's highest recorded yields. In line with other crops in Saudi Arabia, the cultivated area and exports of potatoes increased over recent years, at the same time that imports were reduced (see table 32). As a consequence, the self-sufficiency ratio of Saudi Arabia in potatoes increased from 5% in 1980 to 65% in 1994.

Farmers in Saudi Arabia enjoy two potato crops per year, and the majority of farmers import virus-free potato seed-tubers from France and the Netherlands (158). The same source indicated that virus infections were detected with French potato seeds. Poorer farmers tend to use consumption potatoes from Lebanon as seedling material, causing epidemic proportions of virus infestations in some areas of Saudi Arabia.

The department of Agricultural Protection of King Saud University conducted a survey on potato diseases in the Kingdom from 1989 to 1993. The survey was carried out as part of KACST project No. 81 (see table 28). It was established that 36% of potato diseases were caused by viral pathogens (204a).

The same KACST project targeted the development of tissue culture protocols for producing virus-free potato mini-tubers which could be further propagated into seed-tubers. The research group succeeded in mastering the technique. Further optimization is needed as only 31.6% of tissue culture propagated plantlets were found to be virus-free (204b).

TABLE 33. POTATO PRODUCTION, EXPORTS AND IMPORTS IN SAUDI ARABIA

Potato	1980	1992	1993	1994
Area harvested (1,000 HA)	-	5	9	9F
Production (1,000 MT)	4	86	167	169F
Exports (1,000 MT)	0.3	3.7	3.5	9.0
Exports (1,000 US$)	111	2,023	1,471	3,204
Imports (1,000 MT)	70	127	116	99
Imports (1,000 US$)	17,531	31,736	27,896	23,343
Self-sufficiency ratio	5%	41%	60%	65%

Sources: FAO, *FAO Yearbook Production 1994*, vol. 48 (Rome), pp. 89-90; *FAO Yearbook, Commerce 1994*, vol. 48, p. 114; and *FAO Trade Yearbook 1981*, vol. 35, p. 137.

Notes: F = FAO estimate

Self-sufficiency is calculated by: $\frac{\text{production}}{\text{(production + imports - exports)}} \times 100$

HA = hectares; MT = Million tons.

The results of the KACST project as well as similar experiences in Egypt, Jordan and the Syrian Arab Republic confirm the impression that the mass-scale production of virus-free potato tubers has considerable potential in Saudi Arabia. Both the quality of tubers and total yields of potato could benefit from tissue culture technology to produce virus-free seed-tubers. The Saudi Arabian Government is actively pursuing

self-sufficiency which has not been achieved so far, indicating that further expansion of potato production is likely.

Current Government support levels for R&D may not allow for developing the technology further towards large-scale production of virus-free mini-tubers. It appears, therefore, that the most likely alternative is to encourage public institutions or private companies to explore commercial development of the technology. Since potato seed tubers are currently imported from France and the Netherlands it is most likely that revenues from sales should easily compensate for investment requirements in this area.

(b) *Date palm biotechnology*

If the potato is an evident crop for commercialization in Saudi Arabia, the micropropagation of the date palm is an even more obvious choice for investments. Chapter II (section B) elaborates on the general advantages of date palm micropropagation as a substitute for the traditional offshoot propagation.

Saudi Arabia alone produces 14.5% of the world's total harvest in dates and is the third largest producer in the world. Almost the entire production of dates is used for home-consumption. The Saudi Arabian market for dates is almost entirely an internal affair. Saudi Arabia is also self-reliant in this traditional crop, indicating the limitations of investment opportunities in date palm micropropagation. Not only should micropropagation offer cheaper plantlets to farmers, but the plantlets should also be of superior quality.

The considerable research efforts by Saudi Arabian institutions to develop micropropagation are a clear recognition of the potential commercial and agricultural benefits to be gained in this area.

Since 1994, date palm growers have had access to micropropagated plantlets through the National Agricultural and Water Research centre. In September 1996 date plantlets began to be sold on a commercial basis. This was exactly 10 years after the first attempt was unsuccessfully launched to start commercial micropropagation of the date palm in Saudi Arabia. Owing to legal restrictions any private commercial initiatives were limited at that time, in an attempt to develop the technology inside the country.

TABLE 34. PRODUCTION OF DATES, EXPORTS AND IMPORTS IN SAUDI ARABIA

Dates	1980	1992	1993	1994
Production (1,000 MT)	377	550	563	564F
Exports (1,000 MT)	12.7	18.4	18.2	16.6
Exports (1,000 US$)	4,673	14,876	25,223	13,618
Imports (1,000 MT)	0.7	0.2	0.2	n.a.
Imports (1,000 US$)	521	110	85	n.a.
Self-sufficiency ratio	103%	103%	103%	103%

Sources: FAO, *FAO Production Yearbook 1994*, vol. 48 (Rome), pp. 41-42; *FAO Yearbook, Commerce 1994*, vol. 48, p. 114; and *FAO Trade Yearbook 1981*, vol. 35, p. 170.

Notes: F = FAO estimate

Self-sufficiency is calculated by: $\frac{\text{production}}{\text{(production + imports - exports)}} \times 100$

MT = Million tons.

Now, one decade later, Saudi Arabian public research institutions have indeed developed the necessary elements which are essential for commercial micropropagation. The Date Palm Research Centre in Hofuf has generated the superior organogenesis protocol. The National Agricultural and Water Research Centre has developed most of the essential requirements for enabling large-scale handling of micropropagated date palms. Despite these successful efforts no joint collaboration has materialized for further commercialization of the technology.

Instead it required the input of foreign technology (from the United States) to start commercial micropropagation of date palm in Saudi Arabia in 1996. The major limitation of this private initiative, as well as of public research institutes, is the inability to guarantee the performance of micropropagated date palms in the field.

The traditional offshoot propagation of the date palm allows farmers to check on the quality of the mother plant to determine the actual price of the plantlet. Micropropagation does not allow for a direct reference to the mother plant. Even if careful documentation enables quick referencing to locate the original germ plasm, micropropagation may lead to genetic variations whereby the homology between parent and plantlet is lost. Only DNA fingerprinting or protein analysis could give a reliable indication of the resemblance between both generations and, therefore, the anticipated quality of the plantlet. None of the institutes in Saudi Arabia, private or public, have developed the essential biotechnology analytical tools to provide a quality guarantee of the micropropagated date palms.

(c) *Policy recommendations for national coordination of biotechnology activities in Saudi Arabia*

With regard to prospects for biotechnology to support potato and date palm production in Saudi Arabia it is clear that there is a need for central registration and coordination of research efforts, technology development and commercialization. Such central coordination may ultimately target large-scale sustainable commercial production and marketing of micropropagated date palms and potato mini-tubers. The most favourable scenario could include Saudi Arabian institutions exporting such seedling material.

Much is needed before such favourable developments can take place. Although no specific biotechnology policies are needed, it is a good idea to develop specific date palm and potato seedling multiplication policies. The elements of such policies dealing with biotechnology should consider all of the following:

(a) Core technologies (e.g. micropropagation protocols, quality control) are to be developed and mastered by Saudi Arabian institutions.

(b) Core technologies are to be assembled into a production line.

(c) Retail, distribution and marketing channels for micropropagated seedling material are to be established.

(d) Information and extension services (e.g. demonstrations, follow-up, quality tests) should guarantee optimal use and application of micropropagated seedling material.

In the case of potato micropropagation, core technologies including a standard protocol for a growth medium and quality tests that can monitor whether the propagated material is virus-free have already been developed at King Saud University. For date palm micropropagation, quality tests that can confirm the superiority of seedlings are still to be developed. In both cases the main challenge remains the successive follow-up indicated in items (b) to (d) above.

In order for all interested actors in Saudi Arabia (e.g. researchers, public and private institutions, investors, farmers and consumers) to benefit, central coordinative mechanisms should target the active involvement of all. A second guiding principle for policies should be to stress the reliance on Saudi Arabian human resources in the cases of date palm and potato micropropagation. Only in such a way can the technology be sustainably transferred to the farmers in Saudi Arabia. The country's fifth plan already supports the latter, so it should not be difficult to incorporate this issue into biotechnology policies for date palm and potato propagation.

Thanks to the grants programmes of KACST, an active involvement of the public research community has already been achieved. They recognize their interests and are capable of expanding research activities in both financial and scientific terms if further incentives are provided.

Such incentives should mainly focus on the establishment of production lines within one or more private or public enterprises. It may seem appealing to use additional grants for such incentives. In the case of potato micropropagation, additional funds are necessary as none are available to enable large-scale micropropagation. However, for date palm micropropagation it may be possible to opt to use existing resources instead of merely providing more grant money. Together the Date Palm Research Centre and the National Agricultural and Water Research Centre have the necessary scientific and technological ingredients to develop a large-scale micropropagation production line without additional funds.

To allow for more and larger operations, the involvement of private investors should be considered by allowing them to establish or fund joint ventures between public research institutes and private companies. Similar to the American biotechnology industry, this would allow a sustainable cross-fertilization between both parties. Of course, by allowing researchers in public service to act as catalysts or even entrepreneurs in such collaborations, it is more likely that investors can be persuaded to take a share of the investments and commercial risks involved. Some negative aspects of such a policy plan can be anticipated. In most industrialized countries, especially the United States, public concerns have been expressed that researchers may neglect some of their duties in public research and scientific education. The receiving end of the technology, which not only includes farmers but also consumers, pays double for development costs: first as taxpayers and secondly by purchasing the commodity.

In most developing countries, and, in this case also in Saudi Arabia, no industry has been fully developed for the micropropagation of the ensure date palm or potato. In other words, for such countries public-private collaboration is a prerequisite in order to ensure the provision of new commodities to the public in the country concerned.

The active involvement of private investors and companies has an added advantage in that this sector is much better equipped to secure retail, distribution and marketing channels for date palm and potato seedlings, especially when such policies go hand in hand with a vision for exploring future possibilities to export such germ plasm.

The above policy suggestions emphasize an increasing role for the private sector and a diminishing role for Government authorities. The opposite is true. Only by setting clear priorities and translating them into central registration and coordination will the newly emerging Saudi Arabian private community be able to respond effectively to new opportunities in this field.

Concerned Government authorities have an even more important role, which is to protect the quality of both the process and the end-product. Investors in non-industrialized countries, Saudi Arabia not excluded, have to focus on short gestation periods, to keep up with the volatility of the market. Therefore newly established private companies will most likely try to cut costs concerned with services to consumers and

quality control of the end-product. For both date palm and potato micropropagation, quality control is not only an essential element of the actual product but is also based on advanced biotechnologies (e.g. ELISA for the potato, and DNA fingerprinting or amino-acid/protein analysis for the date palm). The development of technologies for quality control and the setting of quality standards, are exclusively Government tasks in the initial phases of establishing a micropropagation industry. Once private companies are well-established, the sector will automatically recognize that sales can only be sustained by keeping the clients happy. In due course, companies will therefore increase investments for quality control and general services to clients.

In any case, the setting of quality standards will remain exclusively in the public domain, as will the provision of non-commercially oriented services to both farmers and consumers. Farmers and the public will want to know the disadvantages of the technology or product which, of course, will not be made easily available by the private sector.

So far, strategic elements for date palm and potato micropropagation in Saudi Arabia have been considered in general terms. The most important question to be settled is which Government authority is to take the responsibility for coordinating action in this field. The King Abdulaziz City for Science and Technology seems to be one of the most likely candidates, as a special Genetic Engineering and Biotechnology Programme was recently initiated there. An obvious drawback is that the mandate of KACST does not extend into the private sector nor into the provision of seedling material to farmers, which is the prime responsibility of the Ministry of Agriculture. A division of tasks seems the most obvious choice, whereby different authorities (e.g. KACST, Ministries of Agriculture and Water, Commerce, Finance and National Economy and Planning) would work in a concerted fashion to allow for implementing the policies necessary in the different areas. Of course, the more Ministries involved, the more difficult it becomes to ensure timely coordination. Some Ministries may not get involved, thereby allowing for a more direct line of communication between the concerned actors. A central coordination unit with multisectoral authoritative powers over the other Government departments should guide the overall process to ensure the effective transfer of the biotechnologies from the public research community, via production lines and marketing channels, to farmers and consumers.

4. *Syrian Arab Republic*

The most noteworthy exception when it comes to policy formulation for organizing biotechnology is the Syrian Arab Republic. Of the four ESCWA member countries surveyed, the Syrian Arab Republic is the only country where in Government body was actively involved in coordination or policy formulation at the country level (see table 22). Although this might appear to be a disadvantage, it allows for flexibility whereby public and private institutions can carry out research and development as well as proceed on establishing production lines and subsequent marketing of products and services related to biotechnology.

For this reason the General Organization for Seed Multiplication has effectively carried out all these required stages for the technology transfer of virus-free potato micropropagation. As GOSM is the sole public organization with a mandate for potato seed production and marketing, all necessary financial and human resources were already effectively used together to allow for fast development and implementation of the required set of techniques.

(a) *Potato biotechnology*

The Syrian Arab Republic recorded similar yield levels as Saudi Arabia over recent years (see table 33). Cultivation area remained roughly the same but export figures increased drastically (see table 35). In case the GOSM manages to optimize the production of seed-tubers and is capable of setting and maintaining quality standards for a low virus-infection rate, the Syrian Arab Republic may consider export of

micropropagated seed-potatoes in future years. Since export channels for consumption potatoes are already established, the same marketing channels could be explored for potato seed-tubers.

TABLE 35. POTATO PRODUCTION, EXPORTS AND IMPORTS IN THE SYRIAN ARAB REPUBLIC

Potato	1980	1992	1993	1994
Area harvested (1,000 HA)	18	24	20	21
Production (1,000 MT)	279	412	361	377
Exports (1,000 MT)	2.6	135.4	115F	110F
Exports (1,000 US$)	1,101	70,369	57,500F	58,000F
Imports (1,000 MT)	8.6	8.0	3.5	4.7
Imports (1,000 US$)	3,440	6,568	2,600	2,800
Self-sufficiency ratio	98%	145%	145%	139%

Sources: FAO, *FAO Production Yearbook 1994*, vol. 48 (Rome), pp. 89-90; *FAO Yearbook, Commerce 1994*, vol. 48, p. 114; and *FAO Trade Yearbook 1981*, vol. 35, p. 137.

Notes: F = FAO estimate
Self sufficiency is calculated by: $\frac{\text{production}}{\text{(production + imports - exports)}} \times 100$
HA = hectares; MT = Million tons.

Once GOSM establishes a foothold on the export market for seed tubers, larger profits can be anticipated. It is here where specific (potato) micropropagation policies at the country level are needed to explore fully such profits in order to establish a micropropagation industry in The Syrian Arab Republic. GOSM does have the mandate for the multiplication and distribution of a range of other crop commodities for which micropropagation techniques may play a supportive role (e.g. cotton, wheat, barley, maize; [see chapter II, section D] see chapter II-D-iv, p. II-25). However, for other crops for which micropropagation (e.g. date palm), and notably micrografting (e.g. citrus, olives, pistachios, almonds) may play a central role for improvement and expansion of the production of seedling material, GOSM lacks the mandate.

(b) *Policy recommendations for national coordination of biotechnology activities in the Syrian Arab Republic*

Policy formulation and implementation is needed to guide expansion of micropropagation techniques to other crop commodities. Policies could try to involve private companies as already noted above with the country cases on Jordan and Saudi Arabia. In any case, more likely in the Syrian Arab Republic, more coordination between crop research institutions and the three ministries involved with planning of public agricultural research (Ministry of Agriculture and Agrarian Reform, Ministry of Economy and External Trade and Ministry of Irrigation) is needed.

At present the Atomic Energy Commission of Syria (AECS) may well be the most suitable agency to take up coordination of R&D as well as technology transfer into the relevant sectors where biotechnology is expected to play a leading role. As a multidisciplinary agency, AECS is already executing and planning biotechnology research in different areas related to agriculture and health. Their multisectoral experience may be employed to implement policies aimed at coordinating promising initiatives in biotechnology transfer.

There are three major policy alternatives for organizing biotechnology research which the Syrian Arab Republic, and, for that matter, most developing countries may wish to explore:

1. The first option is a focus on the development and adaption of generic biotechnologies (e.g. plant tissue culture, fermentation), requiring limited inputs with relatively good perspectives for a return on investments.

Examples:

(a) Plant tissue culture (leading technology);
(b) Fermentation (leading - supportive technology);
(c) Production of pathogen detection kits based on polyclonal antibodies (supportive technology).

2. A second policy option may be to join the international contest in establishing advanced biotechnologies within the NARS of the Syrian Arab Republic, requiring large investments during a great many years.

Examples:

(a) Production of transgenic plants (leading technology);
(b) Production of plant metabolites by plant cell culture (leading technology);
(c) Production of pathogen detection kits based on monoclonal antibodies (supportive technology);
(d) Development of molecular selection markers (supportive technology).

3. A third option is not so much concerned with the specific nature of biotechnologies but would focus on the status of specific crops. Depending on input requirements, crop value and the potential of research success, policies could stimulate the use of generic biotechnologies in the case of low-input low-output crop production systems (see box 4). Advanced biotechnologies could be stimulated in the case of high-input high-output crop production systems (see box 5). Of course, the development of advanced biotechnologies in selected areas should be preceded by the mastering of the supportive generic technologies.

BOX 4. CROPS SUITABLE FOR GENERIC BIOTECHNOLOGIES

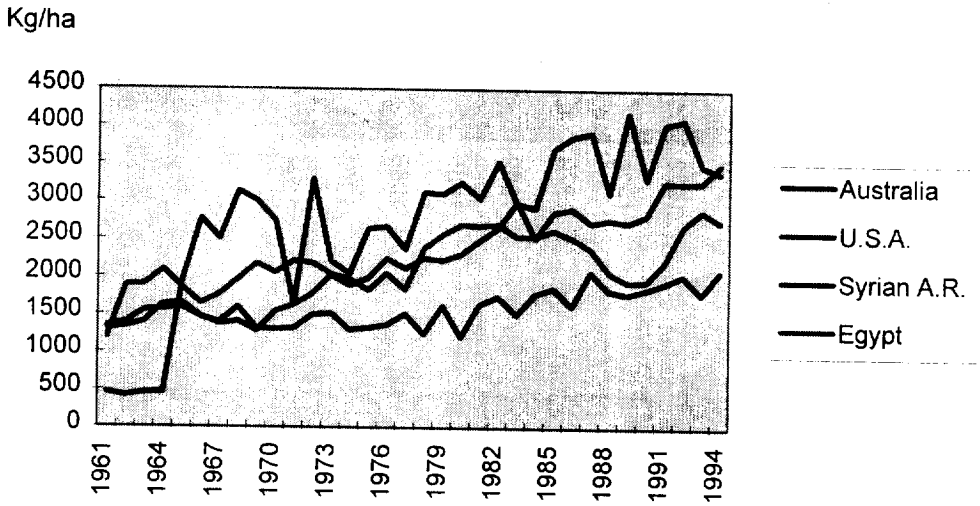
a/ Potatoes (production of virus-free tubers)
b/ Wheat (induction and selection of salt- and drought-tolerant lines of wheat by plant tissue culture)
c/ Barley (double haploid production for breeding purposes)
d/ Date palm (micropropagation)
e/ Citrus (micrografting)
f/ Olives, grapes, pistachios, almonds, dry legumes.

The third policy option above may be the most suitable in the case of the Syrian Arab Republic. For instance, the country has achieved the highest yields in cotton worldwide (see figure IV-6) illustrating the efficiency and resources of the cotton research system in Syria. More advanced biotechnologies could well be suitable in the case of cotton. Other agricultural commodities like barley and potato (see figures IX and X) have very low yields in comparison with maximum yield in other countries. For these low-input low-output commodities a more cautious approach in promoting biotechnologies may be more relevant.

BOX 5. CROPS WARRANTING ADVANCED BIOTECHNOLOGIES

Predominantly cotton and to a lesser degree tobacco and sugar beet are high-input high-output crops. The Syrian Arab Republic has developed an active, and largely export-oriented, textile and clothing industry. Both cotton and tobacco can rely on strong supportive national agricultural research frameworks. Sugar beet production was encouraged by the Government in 1984 in order to supply the domestic sugar-refining industry. Policy pricing in later years caused a drastic reduction in production in later years (178).

Figure VIII. Productivity of seed cotton in Australia, Egypt, the Syrian Arab Republic and the United States

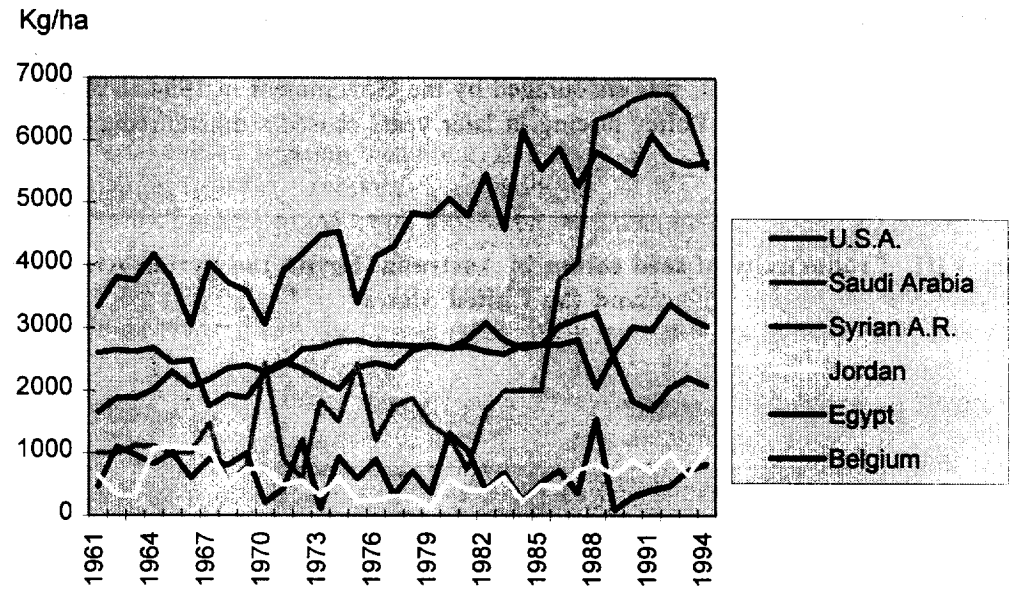


Source: FAO, Agrostat, 1995.

Concerning low-input, low-output crops it must be noted that the Syrian Arab Republic is benefiting considerably from collaboration with ICARDA, as a major part of ICARDA research activities is executed in the Syrian Arab Republic itself. The crop varieties that are officially released by ICARDA are, therefore, well suited to the prevalent ecological conditions in the country. The mandate crops of ICARDA (barley, wheat, chickpea and lentil), may be targeted by Syrian public research institutes to benefit from ICARDA experience. The Atomic Energy Commission has already adopted such policies, as can be seen from its research activities in barley. The Syrian Arab Republic may benefit even more from ICARDA if it were to tighten collaboration agreements concerning the training of human resources. Not only can Syrian research institutes sponsor training of their research personnel on the ICARDA premises in Latakia, but ICARDA research staff may be persuaded to give some training or advice to the country's public research.

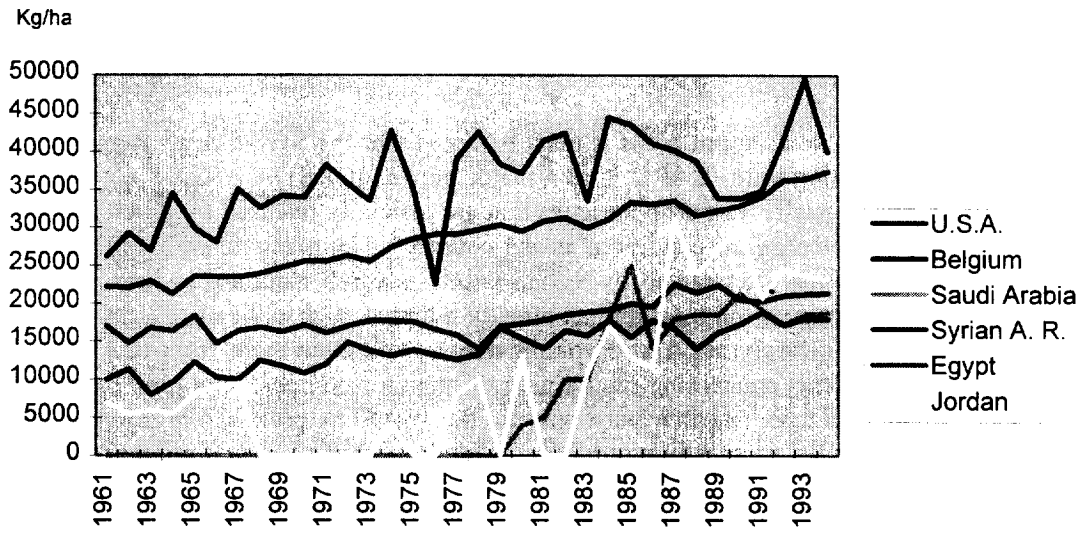
Regional and international collaboration may always be favourable for establishing biotechnology research capacities. It must be emphasized, however, that an over-reliance on resources from outside can endanger the sustainability of local research capacities at both the institutional and the country level.

Figure IX. Productivity of barley in selected ESCWA member countries, Belgium and the United States



Source: FAO, Agrostat, 1995.

Figure X. Productivity of potatoes in selected ESCWA member countries, Belgium and the United States



Source: FAO, Agrostat, 1995.

PART TWO

BIOTECHNOLOGIES FOR INDUSTRY AND MEDICINE

V. ENZYME AND BIOREACTOR TECHNOLOGY

Introduction

Biotechnologies are increasingly playing a key role in industry and medicine. In fact, investments and sales in these biotechnology sectors surpass those in the agriculture sector by far. By comparison, estimates of sales by United States firms of pharmaceutical and diagnostics biotechnology products amounted to US$ 1.5 billion in 1989, compared with US$ 50 million for biotechnology products in the agricultural sector (168a). This trend is also reflected in the areas of primary focus reported by biotechnology companies based in the United States and the United Kingdom. In both countries biotechnology business activities in agriculture account for 17.5% in case of the United States and only 5.7% in the case of the United Kingdom (see table 36).

TABLE 36. MAIN AREAS OF BIOTECHNOLOGY BUSINESS IN THE UNITED KINGDOM AND THE UNITED STATES, 1987

Main area of biotechnology business	United Kingdom (%)*	United States (%)*
Human Health Care	22.9	23.5
Equipment	39.2	3.0
Environmental Control	18.1	1.5
Chemicals	3.0	14.0
Plant Agriculture	3.7	10.5
Animal Agriculture	2.0	7.0
Other**	11.1	40.5

Sources: M. Auramovic, *An Affordable Development. Biotechnology, Economics and the Implications for the Third World* (London, Zed Books, 1996), pp. 16-17 and R. Faulkner Oakey and others, *New Firms in the Biotechnology Industry: their Contribution to Innovation and Growth* (London and New York, Printer Publishers, 1990), p. 41.

* Percentage indicates number of replies of companies compared to the total.
** Other includes: Food, Diagnostics, Reagents and Cell Culture.

Because of the importance of biotechnologies to other sectors, Part two of this study will offer an overview of prevailing biotechnologies and their applications in non-agriculture sectors. Leading biotechnologies in medicine and the industry sectors are mostly applications of enzymes and bioreactor technologies, which will be reviewed in this chapter.

The processing of biological materials and other substrate using biological agents such as cells, enzymes, or antibodies is the central domain of biochemical engineering. When such processes are optimized at a larger scale in a biological reactor, this falls within the area of bioreactor technology. Bioreactor technology holds considerable potential for industrial processing and production of many product groups (e.g.ingredients for foods, detergents, pharmaceuticals, fuel, waste treatment, fine chemicals). This chapter provides an overview of possibilities, requirements and limitations to support government planning and policy activities for bioreactor technology.

Wastewater treatment is especially important for the ESCWA region as it is concerned with one of the most crucial resources and may be used to control pollution due to chemical and household wastes. Bioreactor designs for wastewater will therefore be reviewed in more detail.

A. Research areas

1. *Enzyme technology*

When using biological reactors or bioreactors, often two tools of living organisms are considered for converting a substrate into a product: enzymes and cells. Enzymes, or biocatalysts, form the first category and individual cells of either micro-organisms, plants or animals form the second. Applied enzyme catalysis is inherently different to cell culture, as cells will multiply, given an appropriate environment, whereas enzymes cannot multiply by themselves. This has major consequences for the techniques involved in both bioreactor types. Therefore, a distinction is made between enzyme technology, whereby only enzymes are used for industrial processing and bioreactor technology, which refers to the use of cell-cultures. As with enzymes, cells can either be the desired end-product (e.g. yeast) or cells can be used for transforming a precursor into a desired product. In the latter case, the enzyme machinery of the cells actually performs the tasks.

Enzymes are isolated from microbial fermentations and animal and plant by-products. Microbial enzymes from bacteria, fungi, and yeasts are by far the most common and account for about 80% of the total industrial enzyme production. Over the past 50 years, enzyme production technology has made significant developments that can be viewed in three phases (113a):

(a) In the first phase, enzyme production was confined largely to those enzymes readily available in large quantities from cheap raw materials. Many useful enzymes were identified but not used commercially because of the high cost of their isolation. As this phase developed, methods of extraction and purification were developed and refined.

(b) The second phase (1965-1985) was founded on expansion and refinement of microbiological skills developed from the traditional fermentation industries. The range of available enzymes increased as advances in microbial physiology demonstrated the means to induce high levels of enzyme production. However, genetic manipulation by mutation and selection, although very important in enzyme production, was unpredictable. Yields of enzymes were generally low in fermentation broths, and extraction processes often proved uneconomic.

(c) The third phase (1985 to the present) has made use of genetic technology for transfer of genes between organisms, and the alteration of genes to improve the properties of the selected enzyme by protein engineering. The expectation is that this third phase will not plateau, and that there will be no restriction on the availability of an enzyme for a particular application.

Enzyme applications can be roughly divided into three areas: diagnostic kits, therapeutic systems, and industrial conversions. Often the same enzyme will find applications in more than one area, as can be seen in table 38, where the major commercially produced enzymes are listed. The following section will provide a brief overview of enzyme applications in diagnostics and industry. Medical applications of enzymes will be reviewed in chapter VI. The section on industrial enzymes contains an overall account of different applications of commercially important enzyme classes.

(a) *Diagnostic enzymes*

The inherent specificity and high catalytic power of enzymes make them valuable tools for biochemical analysis in industry and medicine, both as direct agents and as signal generators in enzyme immuno-assay systems. The majority of enzymes used for analysis are derived from animal and plant sources and are used in:

- Clinical biochemistry;
- Food quality control and safety assessment;
- Industrial process monitoring;
- Environmental monitoring;
- Toxicology tests.

The role of enzymes as diagnostics is based on their capacity to catalyse specifically the reactions of one analyte. Detection of the reaction can take place by colourimetry, the formation or disappearance of NADH, the generation of light or ultraviolet detection. Box 6 gives an impression of the many analytes which can be quantitatively analyzed with diagnostic enzymes. The market perspectives for diagnostic applications in medicine will be reviewed in chapter VI.

BOX 6. ANALYTES SUITABLE FOR DETECTION BY ENZYMES

Acetic acid	Ethanol, Methanol	Creatine
Citric acid	Glycerol	Triglycerides
Lactic acid	Acetaldehyde	Amino acids
Pyruvic acid	Malic acid	Phospholipids
Ascorbic acid	Gluconic acid	Ketones
Formaldehyde	Glucose, Fructose etc.	NAD(P)H
Urea	Phenols	Phosphate
Uric acid	Cholesterol	ATP

Source: J.S. Dardick, "*An Introduction to Industrial Biocatalysis*" in *Biocatalysts for Industry* (New York and London, Plenum Press, 1991), pp. 11-12.

Enzyme-based diagnostics have reached the market-place and are readily available for determination of blood glucose and blood cholesterol. Over 60 enzymes have been identified and characterized for use in analytical applications, but very few are available in commercial quantities. Most of the enzymes are required in quantities of tens of kilograms per year (worldwide) with the exception of glucose oxidase which had reached demand levels of hundreds of kilograms per year in 1995 (113c).

(b) *Industrial enzymes*

On a global scale, the industrial enzyme market is reported to be the greatest compared with diagnostic and medical enzyme markets. Total sales in 1995 accounted for US$ 880 million in Europe and the United States combined (see table 37) with detergents for the home laundry market as the leading area. The commercial outlook for industrial enzymes is very strong, and annual sales of over US$ 1 billion are expected for the next five years.

The unique catalytic capabilities of enzymes have been used for thousands of years in traditional industries such as those for making wine, beer, bread and cheese. Today these industries are responsible for almost half of the current use of enzymes. Many useful industrial enzyme preparations are not highly purified. They contain a number of enzymes with different catalytic functions and, under most conditions, are not used with anything approaching either a pure substrate or medium.

Certain applications of enzymes, however, demand the use of relatively pure extracts. For example, glucose oxidase for desugaring of eggs must be free of any protein-splitting enzymes, and proteolytic enzymes injected into animals for meat tenderizing just before slaughter must not contain any compounds that would cause a serious physiological reaction. Other enzymes requiring relatively high purity include enzymes in clinical diagnosis and some enzymes in food-processing. A classification of different useful enzymes is presented in box 7.

TABLE 37. ESTIMATES OF TRENDS IN INDUSTRIAL ENZYME MARKETS (UNITED STATES AND EUROPE) TO THE YEAR 2000
(Millions of US dollars)

Area	1985	1990	1995	2000	TOTAL
Detergents	110	170	280	400	960
Food	100	160	240	370	870
Beverages	100	160	200	320	780
Other	60	100	160	320	640
TOTAL	370	590	880	1,410	3,250

Source: G.F. Bickerstaff, "Review: Impact of genetic technology on enzyme technology," *The Genetic Engineer and Biotechnologist*, vol. 15, No. 1, Journals, Oxford Ltd., pp. 13-14.

(c) *Hydrolases*

The main class of useful enzymes for processing at an industrial scale is hydrolytic enzymes or *hydrolases*. Their primary function is to catalyse the hydrolysis of a variety of compounds including esters and lipids, thiolesters, phosphates, sulphates, proteins and glycosides. The action of hydrolytic enzymes is important not only in obvious macroscopic degradations such as food spoilage, starch thinning, and waste treatment, but also in the chemistry of ripening picked green fruit, self-lysis of dead whole cells, desirable aging of meat, curing cheeses, preventing beer haze, texturing candies, treating wounds, and desizing textiles.

The most important commercially used hydrolases are amylases, which can hydrolyze the glycosidic bonds in starch and related glucose-containing compounds into smaller saccharide units. Table 38 indicates the applications of amylase preparations.

5. *Amylases*

The sources of amylases are numerous. This is not surprising since starch is a common form of carbon fuel for many life forms. Amylases are produced by a number of bacteria and molds. Commercial amylase preparations used in human foods are normally obtained from grains, notably barley, wheat, rye, oats, maize,

sorghum and rice. The ratio of saccharifying to liquefying enzyme activity depends not only on the particular grain but also upon whether the grain is germinated.

BOX 7. MAJOR CLASSES OF ENZYMES

Enzymes are always derived from living sources and can either be the desired end-product of a cell culture (cultivated in a bioreactor) which can then be used in a specific application or the enzymes can be used in a reactor to convert a substrate into a product. Although all living cells produce enzymes, one of the three sources - plant, animal, or microbial - may be favoured for a given enzyme or utilization. For example, some enzymes may be available only from animal sources. Enzymes obtained from animals, however, may be relatively expensive (e.g., rennin from calf's stomach for cheese manufacturing), and may depend on other markets (e.g. demand for lamb or beef), for their availability. While some plant enzymes are relatively easy to obtain (papain from papaya), their supply is also governed by food demands. Microbial enzymes are produced by methods which can be scaled up easily.

As enzymes are the driving force of metabolism of all organisms, there are thousands of different kinds of enzymes. Many of these have been given common names. A rational naming and numbering system has been devised by the Enzyme Commission of the International Union of Biochemistry. Enzymes are divided into six major classes, with subgroups to define their functions more precisely. The major classes are:

1. *Oxidoreductases* - catalyse oxidation/reduction reactions.
2. *Transferases* - catalyse transfer of molecular groups from one molecule to another.
3. *Hydrolases* - catalyse hydrolytic cleavage.
4. *Lyases* - catalyse removal of a group from or addition of a group to a double bond, or other cleavages involving electronic rearrangement.
5. *Isomerases* - catalyse intramolecular rearrangement.
6. *Ligases* - catalyse reactions in which two molecules are joined.

In the production of malt (softened, germinated barley) for brewing, the ungerminated seeds are exposed to favourable temperature and humidity conditions so that rapid germination occurs, with resulting large increase in α-amylase. The germinated barley is then kiln-dried slowly; this halts all enzyme activity without irreversible inactivation. The dried malt preparation is then ground, and its enormous liquefying and saccharifying power (to convert starches to fermentable sugars) is utilized in the subsequent yeast fermentation.

TABLE 38. COMMERCIAL IMPORTANCE AND APPLICATIONS OF SELECTED ENZYMES

Name	Source	Application	Commercial Importance
Diastase	Malt	Digestive aid; supplement to bread; syrup	+++
Amylase	Bacillus subtilis	Desizing textiles; syrup; alcohol fermentation industry; glucose production	+++
Acid-resistant amylase	Aspergillus niger	Digestive aid	+
Amyloglucosidase	Rhizopus niveus, A. niger, Endomycopsis fibuliger	Glucose production	+++
Trypsin	Animal pancreas	Medical uses; meat tenderizers; beer haze removal	+++
Pepsin	Animal stomach	Digestive aid; meat tenderizer	+++
α-Chymotrypsin	Animal stomach	Medical uses	*
Rennin	Calf stomach	Cheese manufacture	+++
Pancreas protease	Animal pancreas	Digestive aid; cleaning; leather bating; dehairing; feed improvement	++
• Papain • Bromelain, ficin	• Papaya • Pineapple, fig	Digestive aid; medical uses; beer haze removal; meat tenderizer	• +++ • ++
Protease	A. oryzae	Flavouring of sake; haze removal in sake	+
Protease	A. niger	Feed, digestive aid	++
Protease	B. subtilis, Streptomyces griseus	Detergents; removal of gelatin from film; fish solubles; meat tenderizer	++
Varidase, Streptokinase	Streptococcus sp.	Medical use	++
Glucose isomerase	Lactobacillus brevis, Bacillus coagulans, Arthrobacter simplex, Actinoplanes missourensis	Isomerization of glucose into fructose	++
Penicillinase	B. subtilis, Bacillus cereus	Removal of penicillin	+
Glucose oxidase	Aspergillus niger	For removal of oxygen or glucose from various foods; dried-egg manufacture	+
Hyaluronidase	Animal, bacteria	Medical use	+
Lipase	Pancreas, mold (Rhizopus)	Digestive aid; flavouring of milk products	+
Cytochrome C	Yeast (Candida)	Medical use	+
Keratinase	Streptomyces fradiae	Removal of hair from hides	+
5'-Phosphodiesterase	Penicillium citrinum, S. griseus, B. subtilis	Inosinic acid and guanylic acid manufacture (5' nucleotides) for *in vitro* DNA synthesis	+++
Microbial rennin	Mucor sp.	Cheese manufacture	++
Naringinase	A. niger	Removal of bitter taste from citrus juice	+

TABLE 38. (*continued*)

Name	Source	Application	Commercial Importance
Laccase	Coriolus versicolor	Drying of lacquer	*
Cellulase	Trichoderma koningi and viride	Digestive aid, cellulose hydrolysis	*
Invertase	Saccharomyces cerevisiae	Confectioneries, to prevent crystallization of sugar; chocolate; high-test molasses	*
Pectinase	Sclerotina libertina, Coniothyrium diplodiella, A. oryzae, A. niger, A. flavus	Increase yield and for clarifying juice, removal of pectin; coffee concentration	+++
Bromelain, ficin	Pineapple, fig	Digestive aid; medical uses; beer haze removal; meat tenderizer	++

Source: J.E. Bailey and D.F. Ollis. *Biochemical Engineering Fundamentals* (McGraw-Hill International Editions, 1986), pp. 158-160.

(+) initiated; (++) established; (+++) international competence;
* not available.

TABLE 39. COMMON APPLICATIONS OF AMYLASE PREPARATIONS

INDUSTRY	USE
Glucose and Syrup	Total or partial hydrolysis of corn starch by amyloglucosidase or α-amylase to give a large quantity of sweeteners.
Brewing	Conversion of crushed grain starch to maltose (a suitable disaccharide substrate for yeast fermentation).
Breadmaking	Leavening: Conversion of sufficient starch to fermentable saccharides needed for carbon dioxide generation.
Fruit juice	Hydrolysis of starch, to dissolve turbidity due to insolubility of starch.
Papermaking	α-amylase action to liquefy starch coatings to a desired viscosity for application to fibers (variable weight papers).
Textiles	Sizing: α-amylase activity to liquefy starch; resulting solution used to strengthen warp threads before weaving. Desizing: α-amylase action to reduce size from woven material so that all threads will dye uniformly and fabric will have desired texture.
Candy	Production of candy of desired softness.

Source: J.E. Bailey and D.F. Ollis, *Biochemical Engineering Fundamentals* (McGraw-Hill International Editions, 1986), p. 164.

(d) *Proteases*

Another important group of hydrolytic enzymes comprises are proteolytic enzymes or proteases. Proteases attack nitrogen-carrying compounds, especially proteins, selectively. The commercial sources of proteases include animals (pancreas) and large plants (sap, juices) as well as yeasts, moulds, and bacteria.

The major uses of free proteases occur in dry cleaning, detergents, meat-processing (tenderization), cheesemaking (rennin only), tanning, silver recovery from photographic film (pepsin), production of digestive aids, and certain medical treatments of inflammations and virulent wounds.

Enzymes were used in laundry aids as early as 1913. During the late 1960s an explosive increase in protease utilization in detergents occurred. The enzymes used facilitate spot removal; they are a mixture of bacterial neutral and alkaline proteases which are active over the pH range of 6.5 to 10 and temperatures from 30° to 60°C. A peak in this enzyme application occurred in 1969 when 30% to 75% of all European detergents and about 40% of detergents in the United States contained enzymes. However, subsequent warnings from the United States. Federal Trade Commission caused concern about health hazards from these preparations, and this enzyme market plummeted in 1970 and 1971. Following retraction of the Trade Commission warning and use of modified manufacturing procedures to minimize enzyme dust formation, a partial recovery followed.

The tenderization of individual meat pieces by commercial tenderizer products depends on proteolytic action of the relatively inexpensive and heat-resistant plant proteases papain and bromelin. Aging of whole meat carcasses prior to cutting and packaging is normally accomplished by controlled partial self-digestion (autolysis) of the bled meat. Ground pancreas preparations from different animal sources contain all the digestive proteases, including trypsin as well as lipases and amylases. These obviously digestive mixtures are useful in dehairing animal hides and for simultaneous removal of other non-collagen protein from hides. Since pepsin itself attacks collagen, the fibrous skin protein which is converted into leather, this proteolytic enzyme is useless in tanning.

In the dairy industries, rennin is the single most important enzyme. It acts by removing a glycopeptide from soluble calcium casein to yield a relatively insoluble calcium paracaseinate, which precipitates to form the desired curd. Shortages of animal rennin have stimulated development of suitable microbial rennin enzymes, and these are now used commercially. Genetic engineering methods have also been applied to produce calf rennin in micro-organisms.

Proteolytic enzymes, especially trypsin, apparently reduce inflammation and swelling associated with internal injuries and infections by dissolving blood clots and extracellular-protein precipitates, by locally activating other body defences which do the same thing, or both. Some severe lung infections resulting in the accumulation of viscous lung deposits have been reduced successfully or eliminated by proteolytic enzyme administration.

A third major group of hydrolytic enzymes are pectic enzymes, mainly extracted from fungi and fruits. Pectic enzymes hydrolyse esterified polygalacturonic acid and are important for the production of fruit and vegetable juices and wine production. Crushing fruits and vegetables yields juices with high viscosities, desirable in the production of tomato and citrus juices but not so much so for apple cider and other fruit juices.

A controlled partial pectin hydrolysis of these juices yields a free-flowing product which retains enough viscosity to prevent undesirable settling of particulate matter. A greater hydrolysis is effected with apple juice: the hydrolysed product is much more easily filtered to yield a clear juice. If the juices are to be used in jelly manufacture, only the pectin esterase is added. The resulting polyacid hydrolysis product is then gelled by precipitation with calcium ions.

In wine production pectic enzymes are added to the crushed grapes, which tends to increase the weight yield of juice. This also allows extraction of greater colour from the grape skin and permits faster filtering and pressing. Later addition to the fermented product again gives a faster subsequent separation of the wine

from the yeast and grape sediment and yields a clear wine with an increased stability. In both the case of wine and juice production, a major use of pectic enzymes is thus the development of a process stream with a desirable viscosity and filterability. The application includes benefits to process economics and the appearance of the product.

2. *Design and analysis of biological reactors*

Bioreactors form the central piece of equipment necessary to accommodate enzymes, micro-organisms and eukaryotic cell-lines. They either serve for the large-scale production of these biological entities or to allow for the conversion of a substrate into a precursor or end-product. It is in the design of bioreactors where the challenges of bioprocess control are to be found, especially since enzymes and cells are forced to operate outside their natural environment.

The design of bioreactors is influenced by two fundamental phenomena; biological reaction kinetics and mass transfer. Biological reaction kinetics describe elements involved in cell growth: uptake of some material from the cell's environment and release of metabolic end-products into the surroundings.

When increasing the size of bioreactors, usually problems related to physical transport phenomena dominate the design of bioreactors. For instance, if a richer supply of carbon nutrients is created, evidently the aerobic cell will be able to utilize them fully only if oxygen can also be maintained at a higher concentration in the direct vicinity of the cell. This situation may call for increased gas-liquid mass transfer of oxygen, which has small solubility in aqueous solutions.

Other mass transfer problems such as liquid-liquid mass transfer of liquid hydrocarbon feedstocks used for single cell protein production and heat transfer phenomena, are other decisive factors which influence the final design of bioreactors. So far, large-scale ideal mixing bioreactors have not been developed. There is, therefore, a considerable need for many scale-up experiments and empiricism in order to arrive at what may be far from optimal processes. The basic bioreactor types, commonly used in biochemical engineering will be described in brief.

(a) *Ideal bioreactors*

There are two basic types of ideal bioreactors: batch reactors and continuous-flow stirred-tank (CSTR) reactors. Many biochemical processes involve batch growth of cell populations. After adding an inoculum of living cells to a liquid medium, nothing (except possibly some gas) is added to the culture or removed from it as growth proceeds. Typically in such a batch reactor, the concentrations of nutrients, cells, and products vary with time as growth proceeds. This is in sharp contrast to CSTR reactors whereby a steady state of these concentrations is reached, by continuous adding of fresh medium and removal of product. The latter approach is practical only when the enzymes are so inexpensive that they are expendable. Use of more costly enzymes requires that they be retained in the reactor or recycled. Ideal bioreactors can only be achieved under small-scale laboratory conditions. Large-scale batch and CSTR reactors typically need design adaptations to allow for more efficient mixing and circulation patterns to optimize the reaction processes.

(b) *Sterilization reactors*

Sterilization of bioreactors is often needed to avoid contamination which might decrease the efficiency of the enzyme or cell culture, or might even cause severe health risks. Requirements for destruction of viable microbes and viruses vary widely depending upon the material and its intended use. In some instances, as with biological wastewater treatment, micro-organisms naturally present in the process fluid are responsible for desirable reactions. Inhibitors for the growth of unwanted organisms are rapidly evolved in alcohol,

vinegar, and silage production, so that here too sterilization requirements are not extreme. Milk pasteurization involves killing most but not all actively growing microbes. More severe treatment of milk is not practised because degradation of desired components results. Trade-offs between destruction of useful compounds and death of unwanted organisms play a major role in choice and design of sterilization equipment.

Pure-culture fermentations, tissue culture, and some food products require more stringent measures. Essentially all contaminating microbial life must be excluded from the system, although the degree of perfection also varies somewhat. Economic considerations might indicate, for example, that a contamination probability of one out of every hundred batches is acceptable for a batch fermentation process. The loss of this one batch is acceptable if it compares favourably with the cost of additional sterilization capacity.

Much more severe requirements are necessary in the canning industry. A single surviving spore of *Clostridium botulinum* may cause lethal contamination, so that virtually complete elimination is required. Typically, a design criterion in this situation specifies that the spore survival probability be reduced to less than 10-12. This example illustrates the importance of small deviations from essentially complete conversion of substrate (spores and vegetative cells) into products (inactive spores, dead cells) in sterilization reactors. Sterilization can be achieved by several means, including radiation, sonication, filtration, heating and chemical addition. Only the last two are widely used in large-scale processes. For instance, in continuous-sterilizer design, direct steam injection and plate heat exchanger are most commonly used.

TABLE 40. DIFFERENT TYPES OF MULTIPHASE BIOREACTORS

Bioreactor type	Examples of applications	Principle	Advantages compared to Packed-Bed reactor
Packed-Bed Bioreactors	• Immobilized enzymes for glucose isomerization. • Selective penicillin hydrolysis. • Selective reactive separation of racemic mixtures of amino acids.	Columns packed with immobilized biocatalyst particles.	
Bubble-Column Bioreactors	• Beer production. • Vinegar manufacture (biological oxidation of ethanol to acetic acid). • Cultivation of microorganisms (SCP) for use as animal feed.	Reactors with a large height to diameter ratio which take the form of columns. Mixing is supplied entirely by forcing compressed gas into the reactor which then rises through the liquid.	• Relatively low capital cost. • Simple mechanical configuration. • Reduced operating costs, based on lower energy requirements.
Fluidized-Bed Bioreactors	• Tower fermentor used for continuous beer production.	Similar to Bubble-column bioreactors with the addition of an additional catalyst phase (i.e. flocculated organisms, pellets of immobilized enzymes or cells).	• Improved contact of the reaction mixture with gases.
Trickle-Bed Bioreactors	• Trickling biological filter for wastewater treatment. • Vinegar manufacture.	Three-phase system containing a packed bed of heterogeneous catalyst and flowing gas and liquid phases.	• Improved contact of the reaction mixture with gases.

Source: J.E. Bailey and D.F. Ollis, *Biochemical Engineering Fundamentals* (McGraw-Hill International Editions, 1986), pp. 609-611.

(c) *Multiphase bioreactors*

Under many circumstances it is valid to treat bioreactors, which almost always contain multiple phases in the form of cells, low solubility substrate or products, gas bubbles, or catalyst particles, as effectively homogeneous. However, there are many cases where it is important to take the multiphase nature of the reactor contents into account when designing the bioreactor or analysing its performance. This is the case, for instance, with bioreactors containing heterogeneous substrate, such as the utilization of starch and cellulose particles, in conversion of steroids, and in growth of paraffinic hydrocarbons. Table 40 lists typical multiphase bioreactors.

3. *Wastewater treatment*

Because of the importance of water resources to the ESCWA region, industrial and domestic wastewater treatment will be reviewed in more detail as an example of applying mixed microbial populations to bioreactor technology. Some characteristics of sewage and industrial wastewater will be explained. Thereafter, the focus will be on the biological components of wastewater treatment processes of sewage. The major processes of wastewater treatment include three major processes:

(a) In primary treatment, the most easily separated contaminants are removed;

(b) In secondary treatment, suspended particles and soluble components, which are mainly of organic nature, are removed;

(c) In tertiary treatment, the removal of the remaining contaminants is accomplished.

Biological treatment of wastewater is mainly confined to the secondary treatment of wastewater, aiming at a total reduction of degradable compounds that would otherwise cause excessive oxygen consumption in the receiving water bodies. The latter part of this section will also consider the removal of more toxic compounds by the use of bioremediation, which is mainly applied as part of tertiary treatment in wastewater treatment plants.

Sewage or domestic wastewater generally consists of substances such as ground garbage, laundry water, and excrement. About 99% of sewage is water. The main component of suspended solids is cellulose, and the bulk of organic matter present is in the form of fatty acids, carbohydrates, and proteins in that order. The bad odour of sewage is mainly due to the protein decomposition under anaerobic conditions. Because of its origins, sewage contains a varied population of soil and intestinal micro-organisms, including aerobes, strict and facultative anaerobes, bacteria, yeasts, molds, and fungi. Since pathogenic organisms and numerous viruses including polioviruses and hepatitis viruses are often present in sewage, it is critically important to isolate drinking water supplies from sewage contamination.

The sewage microbial populations provide a continuous mixed-culture inoculum which is used for both the biological treatment processes and to analyse the degree of cleanliness of the wastewater. Basically, the more micro-organisms are present in wastewater, the more pollutants, acting as a substrate, are also present.

The most commonly used index which measures this sewage strength or concentration is the biochemical oxygen demand (BOD). It is equal to the amount of dissolved oxygen which is consumed by a sewage incubated for a specified number of days at 20° C. Originally devised in 1898 by the British Royal Commission on Sewage Disposal, this test was chosen to simulate the conditions of a stream and to provide a relatively direct measure of one of the most damaging effects of sewage discharge: depletion of dissolved oxygen in the receiving waters.

A lowered dissolved-oxygen value can quickly lead to death of many aerobic organisms and animals; the result may be a smelly river contaminated with pathogenic microbes. In table 40, typical values of characteristic parameters for the influent and effluent streams of a sewage treatment plant are indicated.

The composition of industrial wastes depends strongly on the source. Table 41 shows that many industrial wastes are far more concentrated than sewage. Those derived from processing hydrocarbon materials often contain toxins such as formaldehyde, ammonia, or cyanide. Two main problems related to industrial wastes are:

(a) They are extremely damaging to living organisms of the receiving water;

(b) They may kill micro-organisms utilized in aerobic and anaerobic waste treatment.

TABLE 41. SOME CHARACTERISTIC PARAMETERS FOR WATER QUALITY OF SEWAGE BEFORE AND AFTER BIOLOGICAL TREATMENT

Parameter	Influent raw sewage	Effluent in an acceptable plant
BOD, mg/L	100-250	5-15
Total Phosphorus, mg/L	6-10	0.2-0.6
Nitrogen, mg/L	20-30	2-5
Suspended solids, mg/L	100-400	10-25

Source: J.E. Bailey and D.F. Ollis, *Biotechnical Engineering Fundamentals*, 2nd edition (McGraw-Hill International Editions, 1986), p. 924.

In the treatment of industrial wastewaters, these chemical toxins often constitute major difficulties in optimizing bioremediation of discharge waters.

Basic designs for wastewater treatment facilities were developed during the nineteenth century as one of the first applications of biotechnology for the maintenance and restoration of environmental quality. Since that time, there have been only minor changes in the fundamental designs of the original sewage treatment plants and the way micro-organisms are used. The main bioreactor types used for treatment of wastewater are the activated-sludge system, the trickling biological filter and anaerobic digestion units.

(a) *Activated sludge system*

The activated sludge system is a continuous-flow aerated biological reactor. The aerobic reactor is closely tied to a sedimentation tank, in which the liquid is clarified. A portion of the sludge collected in the sedimentation tank is usually recycled to the biological reactor, providing a continuous sludge inoculation.

A common bacterium in the activated-sludge population is *Zoogloea ramigera.* Perhaps the most important characteristic of this organism and others in the sludge is their propensity for synthesizing and secreting a polysaccharide gel. Because of this gel, the microbes tend to agglomerate into flocs, which are called activated sludge.

A special property of activated sludge is its high affinity for suspended solids, including colloidal materials. Thus the initial step in removing suspended solids from the wastewater is attachment to the floc. Following this, biodegradable components of the adsorbed particulates undergo oxidation by floc organisms.

In addition to possessing the necessary adsorbent and metabolic qualities, a good sludge should settle rapidly. This settling will avoid bulking, or overflow of the sludge into the effluent. Poor sludge often contains high concentrations of filamentous bacteria and flagellate protozoa, whereas healthy sludge contains more stalked ciliated protozoa. This latter species preys on free, i.e., inflocculated, bacteria and thereby clarifies the effluent.

(b) *Trickling biological filter*

The so-called trickling or percolating biological filter is a popular alternative to the activated-sludge process. Here a film or slime of micro-organisms lives on solid packing which loosely fills a vessel designed to permit air to enter the lower portion of the bed. Liquid waste is fed to the top of the bed which trickles over the slime-covered packing in films sufficiently thin for the oxygen to supply aerobic organisms in the outer surface of the microbial film.

TABLE 42. COMPARATIVE CHARACTERISTICS OF INDUSTRIAL WASTEWATER AND DOMESTIC SEWAGE

Type of waste	Main pollutants	BOD, mg/L after 5 days of incubation
Abattoir	Suspended solids, protein	2,600
Brewery (bottle washing)	Carbohydrate, protein	550
Cannery (meat)	Suspended solids, fat, protein	8,000
Chemical plant	Suspended solids, extremes of acidity or alkalinity, organic chemicals	500
Distillery	Suspended solids, carbohydrate, protein	7,000
Dairy	Carbohydrate, fat, protein	600
Domestic Sewage	Suspended solids, oil-grease, carbohydrate, protein	350
Fermentation Industry	Suspended solids, carbohydrate, protein	4,560
Grain-washing	Suspended solids, carbohydrate	1,500
Petroleum refinery	Phenols, hydrocarbons, sulphur compounds	840
Pulp mill	Suspended solids, carbohydrate, lignin, sulphate	25,000
Starch reduction of flour	Suspended solids, carbohydrate, protein	12,000
Tannery	Suspended solids, proteins, sulphide	2,300

Source: J.E. Bailey and D.F. Ollis, *Biotechnical Engineering Fundamentals*, 2nd edition (McGraw - Hill International Editions, 1986), pp. 609-611.

Unlike the activated-sludge process, which often receives forced aeration, air is circulated through the trickling filter by natural convection. As the wastewater trickles down the bed, the composition gradually changes as different components are removed by different micro-organisms. Organisms best suited to utilize the feed sewage as a nutrient predominate at the top of the bed, like fungi and free-swimming ciliated protozoa. In the lower portions of the filter live stalked ciliate protozoa and nitrifying bacteria. Higher animals are also among the inhabitants of biological filters, with worms and fly larvae the major populations.

These animals graze on the slime film which grows on the filter packing, and control of their populations is an important factor in filter operation.

The spatial segregation of organisms in a biological filter provides an opportunity for each species to adapt fully to its immediate environment. Because of this, low-rate biological filters usually provide clearer and more highly nitrified effluents than activated-sludge systems do. Experience has shown that filters are less sensitive to shock loads of toxic substances than activated-sludge processes. Costs of activated-sludge systems, however, are generally lower. A comparison between both bioreactor types, together with the most promising anaerobic digestion unit is shown in table 43. The choice between the two processes requires careful consideration of waste characteristics, costs and environmental standards.

TABLE 43. COMPARISON BETWEEN TRICKLING-FILTER AND ACTIVATED-SLUDGE WATER TREATMENT SYSTEMS

Item	Trickling biological filter	Activated-sludge system	Anaerobic sludge blanket
Capital costs	High	Medium	Low
Operating costs	Medium	High	Low
Space requirements	High	Medium	Low
Aeration control	Partial	Complete	None
Temperature control	Difficult due to large heat losses	Complete; heat losses small	Difficult
Sensitivity to variations in applied feed concentrations	Fairly insensitive but slow to recover if upset	More sensitive but recovery quite rapid	Medium sensitive, recovery quite rapid
Clarity of final effluent	Good	Not as good	Bad
odour nuisance	High	Low	Low

Sources: J.E. Bailey and D.F. Ollis, *Biochemical Engineering Fundamentals*, 2nd edition (McGraw-Hill International Editions, 1986), pp. 943-944; and G. Lettinga and others, Anaerobic Waste Water Treatment as an Appropriate Technology for Developing Countries, Trib. Cebedeav (Liège, Belgium) (1987), No. 519, pp. 22-23.

(c) *Anaerobic digestion unit*

The third bioreactor type used in wastewater treatment, mainly in conjunction with the previously described bioreactor systems, is the anaerobic digestion unit which converts organic matter into methane and carbon dioxide. Anaerobic digestion is especially beneficial for bioremediation of wastewater containing high concentrations of fermentable organic compounds. A simplified schematic of the overall mechanism of anaerobic digestion, which involves a multitude of microbial species, is indicated in figure XI.

Energy costs for this bioreactor type are usually high as anaerobic digestion is more rapid at higher temperatures (40° to 60° C). Fortunately, the anaerobic digestion process produces a fuel which can be used to reduce energy costs for the wastewater treatment plant. In some instances, the methane produced by anaerobic waste treatment is used outside the plant for heating and power. Since the gas mixture produced by anaerobic digestion reactors is lower in quality than natural gas, it has not been such an attractive product in areas where natural gas supplies are plentiful, as in the ESCWA region.

As a result of anaerobic digestion, the sludge is in much better condition for further treatment. First, the organic sludge solids are reduced by as much as 50% to 60%. Moreover, the composition of the sludge

is profoundly changed. Digested sludge, therefore, is much less polluting than raw sludge, and it is also easier to dewater. After dewatering, which is often accomplished with rotary-drum vacuum filtration, the sludge is dried further, then spread on land as a fertilizer, dumped, or incinerated.

The most promising anaerobic wastewater treatment system is the Upflow Anaerobic Sludge Blanket reactor (UASB reactor). The increasing popularity of UASB reactors must be attributed to their significantly higher loading potentials and lower capital costs (109a), when compared with other aerobic and anaerobic systems. The UASB concept was first applied in the United States in the 1950s and in South Africa in the early 1970s. More than 20 UASB plants were built in Brazil during the 1980s (109c).

Figure XI. Major biochemical reactions taking place in anaerobic digestion unit of a wastewater treatment plant

1/ Insoluble organics $\rightarrow$ Soluble organics
(liquefaction by extracellular hydrolases)

2/ Soluble organics $\rightarrow$ (a) Bacterial cells.
(acid producing bacteria) (b) Other products.
(c) Volatile acids + CO_2 + H_2

3/ (c) Volatile acids $\rightarrow$ CH_4 + CO_2 + bacterial cells
(gasification by methane producing bacteria)

Sources: J.E. Bailey and D.F. Ollis, *Biochemical Engineering Fundamentals*, 2nd edition (McGran-Hill International Editions, 1986), p. 803.

Most anaerobic treatment systems require two reactors, i.e. one for acidification and one for methanogenesis. Usually this is not the case for UASB reactors as both reactions can take place in the same reactor. This simplifies the UASB considerably and makes it cheaper to develop. This is especially valid for hot climates as anaerobic treatment generally requires higher temperatures (above 20° C) to operate efficiently.

The most noteworthy advantage of the UASB reactor over other systems is that it can be applied on a very small scale and a very large scale. Conventional wastewater treatment plants are usually only affordable on a large scale requiring an elaborate and expensive sewerage network. Anaerobic treatment units also allow for an easy expansion of existing facilities when the supply of sewage increases.

(d) *Bioremediation*

Biotechnology has a special new role to play in wastewater treatment plants in degrading industrial xenobiotic (not naturally occurring) pollutants such as halocarbons (e.g. DDT). This process is called bioremediation, whereby polluting substances are converted to carbon dioxide and microbial biomass. In most cases, bioremediation relies upon naturally occurring micro-organisms that are indigenous to the contaminated site.

For bioreactor design, the major implication of bioremediation is varying the oxygen concentration. Conventional wastewater reactors traditionally favour aerobic bacteria whereby organic compounds are easily degraded. Some micro-organisms, requiring anaerobic conditions, do have metabolic pathways to convert halogenated hydrocarbons to compounds which can then be degraded under aerobic conditions. By alternating aerobic and anaerobic conditions in wastewater treatment plants, the degradation of halogenated compounds, such as PCBs (polychlorinated biphenyls) can be realized (120a).

In today's wastewater treatment plants, inorganic compounds, such as phosphates can also be removed. This is achieved by first growing specific bacteria under anoxic (free of air) conditions, where they accumulate poly-hydroxybutyrate. If these same bacteria with the accumulated poly-ß-hydroxybutyrate are subsequently grown under aerobic conditions, they will take up large amounts of phosphate and incorporate it into polyphosphate, thereby removing it from the wastewater.

Major limitations for bioremediation are that degradation rates for more complex xenobiotic compounds are considerably prolonged. This problem is specifically concurrent with the bioremediation of oil spills. The two approaches taken for the bioremediation of petroleum pollutants are the addition of micro-organisms (seeding) that are able to degrade hydrocarbons and the modification of the environment, for example, by adding fertilizers or by aerating the contaminated site.

Because hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater and soil habitats, adding seed cultures has proved less promising for treating oil spills than adding fertilizers and ensuring adequate aeration.

Nevertheless, many companies are developing and marketing hydrocarbon-degrading seed cultures. Most micro-organisms considered for seeding are obtained from cultures obtained from previously contaminated sites. Some of these seed cultures may be useful for treating heavy oils that contain hydrocarbons that are relatively resistant to degradation, but seed cultures are likely to be of little benefit for the treatment of the bulk of petroleum contaminants. As the use of micro-organism inoculants is not directly beneficial, so is the use of genetic engineering in bioremediation. This is further hampered by international regulations that block the application of genetically engineered organisms for the purpose of *in situ* bioremediation.

So far, the largest application for bioremediation of oil spills has been after the wrecking of the Exxon Valdez in Alaska (120b). Slow release fertilizers were used to treat hundreds of miles of contaminated shorelines. Results from the use of fertilizer solutions demonstrated that oil biodegradation rates in Alaska were limited by the availability of nitrogen and phosphorous, and that the clean appearance of rock surfaces following fertilizer bioremediation treatment was directly caused by biodegradation. Rates of stimulation by bioremediation with fertilizers typically were about 3 to 5 times the natural rates of oil biodegradation.

Greater stimulation might be achieved by higher levels of nutrient addition, but this could risk ecological side-effects such as toxicity to marine life and eutrophication with associated algal blooms. In Alaska, the addition of fertilizers caused no eutrophication, no acute toxicity to sensitive marine test species, and did not cause the release of undegraded oil residues from the beaches.

Another *in situ* application of micro-organisms for biodegradation of oil is the use of micro-organisms to enhance oil recovery. Conventional oil production technologies recover only approximately one third of the original known in-place oil following water flooding. Technologies to produce this remaining oil offer enormous economic potential through the development of new and cost-effective methods.

One method to explore these inaccessible oil reserves is microbial enhanced oil recovery (MEOR). The method involves the injection of micro-organisms along with appropriate nutrients to support microbial growth for the production of metabolic products considered beneficial to trapped oil displacement. Depending on geological conditions, positive effects of injected microorganisms may include:

(a) Acids for reservoir rock modification, improved formation porosity and permeability, and carbonate rock dissolution;

TABLE 44. APPROXIMATE VALUE OF OIL NOT ECONOMICALLY RECOVERABLE BY PRESENT TECHNOLOGY

Source region	Estimated value of oil unrecoverable by present technology ($ x 10^{12}, 1987 prices)
Africa	1.9
Asia-Pacific	0.6
Central Asia	2.9
Middle East	12.4
North & South America	4.1
Western Europe	0.8
TOTAL	22.7

Source: *Biotechnology: the Science and the Business*, V. Moses and R.E. Cape, eds. (Harwood Academic Publishers), 1991.

(b) Biomass accumulation for selective and non-selective plugging of channels and fractures, wetting of rock surfaces, and oil emulsification through bacterial adherence;

(c) Gas production (CO_2, CH_4, H_2) for reservoir repressurization, oil swelling, and viscosity reduction;

(d) Solvents production (ethanol, propanols, butanols, acetone) for oil solubilization;

(e) Biosurfactants production for lowering of interfacial tension and emulsification;

(f) Biopolymer production for plugging and mobility control.

One major negative drawback of MEOR is the stimulation of nascent microbial populations within the oil reservoir, which could result in the biotransformation of good oil (high concentration of low molecular weight hydrocarbons) to poor quality oil (high concentration of high molecular weight asphaltenes and resins). Successes in using MEOR, resulting in increases of oil recovery, have been shown to be highly variable, ranging from 0% to 200% in more than 300 trials since 1953 (121a). Achievements seem to be dependent on a multitude of factors such as geological conditions, kinds of micro-organisms used and the kind of oil present in the reservoir. As the outcome of using MEOR is still unpredictable, additional research is necessary.

B. Financial arrangements and developments

Economics considerations of bioreactor technologies are complex. In order to ensure relevance it is essential to take into account the particularities of the sectors involved. While cost estimates may often be identified with some certitude, the need to analyse marketing prospects for a specific product, e.g. enzyme, monoclonal antibody or vaccine, presents a more important challenge.

1. *Cost factors*

The use of bioreactors for the large-scale use of enzymes or for fermentation purposes requires large investments. For instance, a wastewater treatment plant for a major city in the United States, comprising an active-sludge system and an anaerobic digestion unit, cost in excess of US$ 100 million in 1986. For a rough analysis of required investments for a fermentation plant, costs are usually divided into: capital costs (bioreaction and product recovery equipment); production costs (e.g. salaries, supplies, maintenance and taxes); and utilities costs (feedstock nutrients, additives, packaging agents and materials, electrical, gas, steam, and water needs). The latter two cost factors depend largely on local economic pricing mechanisms. Approximate costs for equipment commonly used in the fermentation industries in 1977 are given in table 45.

Table 45. Typical equipment costs for a fermentation plant in 1977
(US dollars)

Fermentors (stainless with coils, baffles, 2.75 atm. rating)	
15 m^3	35,000
113 m^3	95,000
225 m^3	165,000
Holding tank (stainless steel)	
190 m^3 silo	37,000
Installed, with instrumentation	60,000
Spray dryers (stainless steel)	
Nominal water removed, kg/h	Installation costs
1800	600,000
3200	1,250,000
4500	1,600,000
(Complete with fans, ducts, power lines, steam preheater, gas burner, dryer chamber, wet collection system, bagging system, buildings)	

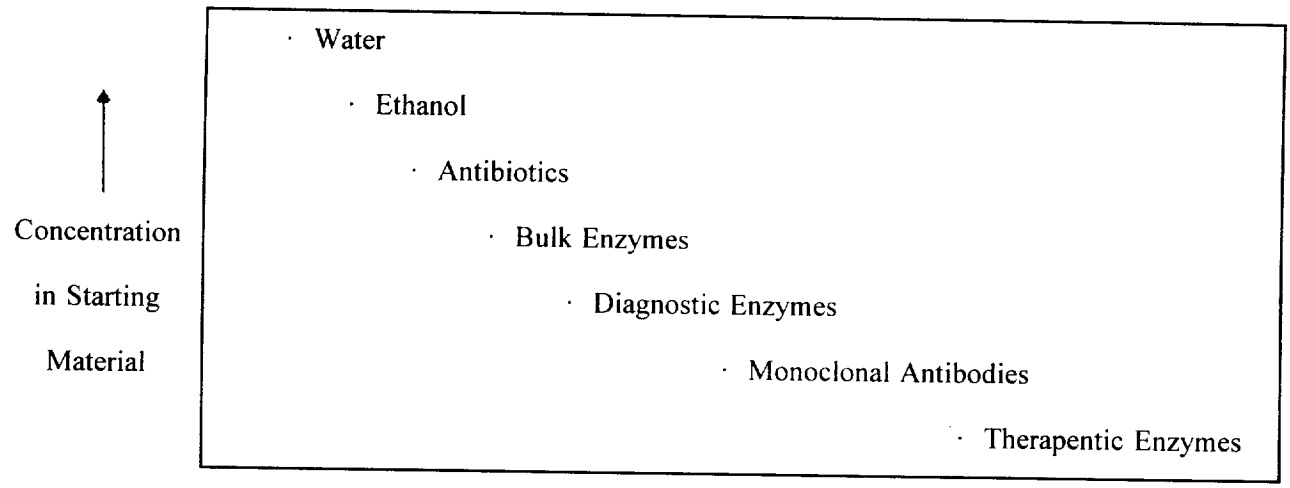
Sources: J.E. Bailey and D.F. Ollis, *Biochemical Engineering Fundamentals*, 2nd edition (McGraw-Hill International Editions, 1986), p. 803.

2. *Fine chemicals*

Enzymes form the biggest single market in bioreactor technology. Currently the industrial enzyme market is the largest, at around US$ 430 million in the United States, with a similar amount in Europe although the medical sector is rapidly catching up from sales of a few highly priced enzymes, such as tissue plasminogen activator (113a). Such enzymes are particularly expensive, because of the high costs of isolation and purification. Figure 12 illustrates the connection between starting product concentration in completed bioreactor medium and the final selling price of the prepared product.

Only rough estimates are available for the cost of research and development to identify promising new enzymes and their subsequent recombination and installation into a new host for mass production. Four research teams with different fields of expertise (analytical chemistry, biochemistry, molecular biology) would be required to execute the operation, taking a total of 6 to 9 years (110a). Each team would be composed of an experienced researcher at post-doctoral level, with graduate research assistants and a technician. Including support staff and accommodation, the annual costs for each team amounted to US$ 160,000 in 1990 (110a). Therefore the total research cost would go as high as US$ 3 million to US$ 6 million in revenue costs alone. Based on these calculations, enzyme-based biotechnology research is only justifiable for foodstuffs or ingredients with an annual turnover of US$ 16 million or more (110a).

Figure XII. Economics-driven connection between selling price (in 1984) and initial product concentration of the completed bioreactor medium



Sources: J.E. Bailey and D.F. Ollis, *Biochemical Engineering Fundamentals*, 2nd edition (McGraw-Hill International Editions, 1986), p. 789.

The principal end-use for industrial enzymes is in detergents for the home laundry market, and almost 60% of industrial enzymes are used in this area. The commercial outlook for industrial enzymes is very strong, and growth expectations in industrialized countries in the next five years are estimated at 50% (113a).

3. *Bulk oxygenates*

Both anaerobic and aerobic processes are suitable for present day bulk oxygenate production. The oldest oxychemical is ethanol, produced in traditional fermented beverage manufacturing. The potential market size for the production of oxygenates is enormous, as illustrated in table 45.

4. *Biogas production*

The comparatively high cost involved in the production of biogas on a larger scale in comparison with prices for other fuel sources is the main limitation in introducing biogas production as an alternative energy source for industries and electricity. Biogas and alcohol production are comparatively more expensive than non-renewable energy sources (see table 47). The production of biogas is, however, a more suitable solution than the production of alcohol as a renewable source of energy.

Although the data of table 46 are relatively old, the observations are further confirmed in a more recent study in 1994: Spelman found that the cost of agricultural raw materials was too high in relation to oil-based products (111a). This is likely to remain unchanged for some time to come. The underlying economic trend is, however, in agriculture's favour as oil is a finite fossil fuel and will thus eventually rise in price while agricultural raw materials are renewable and are becoming progressively cheaper in relation to oil (111b). For instance, in 1990 one ton of oil bought four times as much wheat as in 1967.

TABLE 46. MARKET SIZE OF OXYCHEMICALS FROM RENEWABLE RESOURCES (INCLUDING FERMENTATION)
(US dollars)

Chemical	1981 U.S. production (million pounds)	1983 price, cents per pound	1981 commercial value (millions of dollars)	Major use or derivative
Ethanol				
Ethylene	28, 867	0.25	8.169	Polyethylene, ethylene oxides
Butadiene	3,046	0.34	1,234	Styrene-butadiene rubber, polybutadiene rubber,
Industrial	1,157	0.275	359	Solvents, ethyl acetate and other esters
Ethylene glycol	4,055	0.27	1,281	Polyethylene terephthalate, antifreeze
Acetic acid	2,706	0.265	511	Vinyl acetate, cellulose acetate
Acetone	2,167	0.31	483	Solvents, methyl, and other methacrylates
Isopropyl alcohol	1,644	0.31	507	Acetone, solvents
Adipic acid	1,210	0.57	653	Nylon 66
Butanol	823	0.335	251	Solvents, butyl acrylate
Acrylic acid	691	0.58	276	Polymers
Methyl ethyl ketone	626	0.37	260	Solvents
Propylene glycol	480	0.44	208	Unsaturated polyester resin
Glycerol	370	0.805	259	Drugs, cosmetics
Citric acid	235	0.75	192	Food, drugs

Sources: J.E. Bailey and D.F. Ollis, *Biochemical Engineering Fundamentals*, 2nd edition (McGraw-Hill International Editions, 1986).

TABLE 47. COMPARATIVE COSTS OF RENEWABLE AND NON-RENEWABLE ENERGY SOURCES IN 1976

Fuel	Raw Material	Process	Cost US$/10^9 J
Alcohol	Cassava tops and tubers	Enzyme hydrolysis	8.4
Alcohol	Eucalyptus	Enzyme hydrolysis	20.1
Methane	Eucalyptus	Bacterial fermentation	5.5
Methane	Cereal straw	Bacterial fermentation	4.2
Kuwait crude oil	-	-	1.25*
Gasoline	-	-	4.45
natural gas	-	-	1.15

Source: A. Barnett and others, *Biogas Technology in the Third World; A Multidisciplinary Review* (Ottawa, 1978, International Development Research Centre).

* Based on US$ 10 per barrel of crude oil.

A 1984 feasibility study made by the Government of Thailand with USAID confirmed that the production of biogas on a larger scale (i.e. 2,500 m^3 or more) was not competitive with fuel oil production and that the period before investors would see a return could investment return periods be as long as 30 years (156).

It is not only price levels that restrict the application of biogas technology to rural areas, where kerosene and natural gas may be more expensive. As high outside temperatures are required (29° C is an ideal temperature), biogas is not a likely alternative to natural oil and gas as a renewable source of energy.

Other renewable sources of energy such as solar, wind, water and nuclear energy are considered more beneficial to industrialized countries. For the ESCWA region, the temperatures would allow for the efficient production of biogas but prices of natural gas and oil are low and supplies are abundant. Although generally the need for energy is the driving force behind the exploration of biogas technology, in Japan the prime force in the propagation of biodigesters is pollution control. The countries with the most experience in biogas technology include China (4.6 million plants in 1989 (157)), India and some South-East Asian countries. In most Asian nations it is the rich who have installed biogas plants. The findings of ILO and ESCAP indicated that the share of biogas in the overall energy balance may remain relatively modest. In India, for example, it is estimated that about 400,000 family plants are established, whereas 10 million rural families, out of a total of 75 million, could potentially support biogas plants (155a).

C. Future developments

Fermentation, or the growth of cell cultures to obtain useful products, is as old as mankind. Cell cultures, however, are less efficient catalysts than enzymes and are, therefore, associated with low product yields, higher nutrition requirements, and undesired side reactions. Clearly enzyme technology holds more promise for future developments in bioreactor technology.

For bioreactor technology, the commercial viability of the overall industrial operation is mainly dependent on the availability of enzymes. During the 1980s, investments in research and development for new applications of enzymes were hampered as every enzyme application that was capable of commercial exploitation was successfully developed.

The use of recombinant DNA technology revitalized the commercial viability of producing certain enzymes and forms the major driving force, at present, for novel applications of enzymes. It is estimated that over 50% of bulk industrial enzymes on the market are derived from organisms that have been subject to genetic engineering (113d). The three earliest recombinant products were human insulin (1979), human growth hormone (1981) and human leukocyte interferon (1981) (106j). These are, however, growth-regulating factors or hormones, and will thus not be further reviewed.

The first recombinant enzyme produced for commercial use was recombinant chymosin in *E. coli*, for cheese production by Pfizer in early 1991 to replace the traditional supply of chymosin, which is extracted from calf stomachs (113e). New areas of enzyme applications have recently appeared, thanks to genetic engineering, in for instance the reduction of pollutants in the pulp and paper industry.

Many studies have demonstrated the value of enzymes such as xylanase in reducing the need for chemicals that generate pollution. Hydrolysis of xylan by xylanases facilitates the separation of lignin and cellulose, and reduces the need for chlorine to bleach the pulp. Use of xylanase could reduce the current output of hundreds of thousands of tons of chlorinated organic compounds into the environment (113e). The isolation of xylanase by extraction from cultures of wood-rotting organisms have proved too expensive for industrial use. Recombinant DNA techniques have dramatically reduced the costs, and allowed the production

of recombinant xylanase for use in the pulp and paper industry. This enzyme can have considerable impact for bioremediation of wastewater as the pulp mill industry is the most polluting industry in terms of biochemical oxygen demand.

Low natural availability of novel enzymes is still a substantial barrier in enzyme isolation technology, but it is fading steadily as possibilities for producing recombinant enzymes have become more widespread. For instance, a company such as Novo Nordisk, based in Denmark, re-examined enzymes with known potential that were previously too uneconomical for commercial development. This has resulted in the commercial production of cyclodextrin glycosyl transferase (CGT). This, in turn, has opened a pathway for large-scale application of cyclodextrins in the food and pharmaceutical industries, for use as emulsifiers and foaming agents and as stabilizing agents for volatile pharmaceutical chemicals, antibiotics, hormones and vitamins.

Increasingly manipulation of genes encoding for enzymes and micro-organisms is leading to an increased array of commercially viable applications in enzyme and bioreactor technology. Enzyme or protein modification by directly incorporating new chemical groups will assist in enlarging opportunities for this industry. The most rapid developments are taking place with medical biotechnologies, a subject which is reviewed in chapter VI.

D. Regional developments

Without exception, all member countries have developed conventional (i.e., without using genetically engineered organisms or molecules) fermentation technologies. Complete biotechnology products which are manufactured in the region include cheese, ethyl alcohol, yoghurt and baker's yeast, as indicated in table 48.

TABLE 48. PRODUCTION FIGURES OF ENZYME AND FERMENTATION-RELATED PRODUCTS IN ESCWA MEMBER COUNTRIES

	Cheese 10^3 Metric Tons			Ethyl Alcohol 10^5 liters			Yoghurt 10^3 Tons	Baker's Yeast 10^3 Tons
Country	1992	1993	1994	1991	1992	1993	1993	1993
Egypt	3.18	3.25	3.34	...	...	...	...	...
Iraq	25.6	25.0	24.7	...	...	...	...	...
Jordan	3.94	4.61	4.61	5.3	4.1	5.0	...	...
Lebanon	13.7	14.0	14.7	...	...	...	...	...
Oman	0.38	0.40	0.41	...	...	...	...	...
Syrian Arab Republic	72.3	74.8	78.6	18	23	21	12	15
Yemen	9.2	9.3	9.2	...	...	...	...	...

Source: FAO, *FAO Yearbook Production 1994*, vol. 48 (Rome), pp. 220-221. The Hashemite Kingdom of Jordan, *Statistical Yearbook 1994*, No. 45 (Amman, Department of Statistics, 1995), p. 153; Central Bureau of Statistics, Syrian Arab Republic, *Statistical Abstract, 47th year* (1994), p. 158 and p. 160; and *Industrial Commodity Statistics Yearbook* (New York, United Nations Statistical Division, 1993).

... No data available or no production.

Most countries in the region have established modest industries for the production of fruit and vegetable juices, utilizing enzymes for thickening, to remove any bitter taste or to dissolve turbidity. Other product groups for which biotechnologies are used in part for the manufacturing process include the canning industry

as an example of sterilization bioreactor technology. Other sectors that are well established in the region for which biotechnologies are used as supportive tools are the leather industry (i.e. enzymes for tanning of skins and hides), alcoholic beverages industry (i.e. fermentation), and the detergent industry (i.e. proteolytic enzymes). Table 49 indicates production figures of these respective sectors in ESCWA member states.

The production of alcoholic beverages and of ethyl alcohol is not established in some Gulf countries because of legislative regulations. The Eastern Mediterranean ESCWA member countries have limited production of alcoholic beverages for local consumption. Some ESCWA member countries have an export-oriented leather industry. Net values of export for hides and skins in the Syrian Arab Republic for instance amounted to US$ 11.4 million in 1994 (136f). Jordan's net leather exports are valued at US$ 4.3 million. Other major leather-producing countries in the region (i.e. Bahrain, Qatar, Saudi Arabia and the United Arab Emirates) are exporting small amounts, whereas Egypt, the biggest skin-and hide-producing country in the region, is importing leather on a net basis, valued at US$ 33 million in 1994 (136).

The tanning industry is well established in most non-industrialized countries, including Western Asia. Since enzymes are widely used in the tanning process (including fleshing and dehairing) the leather industry constitutes a considerable market for enzyme technology.

According to a 1993 United Nations study (108a), modern wastewater reuse facilities are currently functioning in seven countries of the ESCWA region. These are Bahrain, Jordan, Kuwait, Oman, Qatar, Saudi Arabia and the United Arab Emirates. In all cases studied, secondary treatment of wastewater was employed, using aerobic digestion such as the activated sludge-system, followed in some cases by rapid sand filtration.

TABLE 49. PRODUCTION FIGURES OF INDUSTRIES IN ESCWA MEMBER COUNTRIES FOR WHICH BIOTECHNOLOGIES (ENZYMES AND FERMENTATION) ARE USED AS A SUPPORTIVE TOOL

	Cattle hides 10^3 metric tons		Sheepskins 10^3 metric tons		Goatskins 10^3 metric tons		Alcoholic beverages 10^5 liters		Detergents 10^3 metric tons
Countries	1993	1994	1993	1994	1993	1994	Beer, 1993	Wine, 1993	1993
Bahrain	0.15	0.15	0.87	0.91	0.23	0.23	...	...	...
Egypt	41.1	39.9	6.5	6.0	6.0	6.3	350	...	64.0
Iraq	4.2	3.9	3.5	3.5	1.1	1.1	...	...	...
Jordan	0.34	0.36	2.1	2.3	0.37	0.37	52.4	2.2	...
Kuwait	0.14	0.14	8.8	8.8	0.06	0.06	n.r.	n.r.	7.0
Lebanon	2.1	2.1	0.81	0.81	0.53	0.53	...	...	...
Oman	0.31	0.31	0.76	0.83	0.47	0.48	n.r.	n.r.	...
Qatar	0.03	0.04	1.6	1.7	0.09	0.09	n.r.	n.r.	...
Saudi Arabia	4.2	4.3	10.5	10.9	4.3	4.4	n.r.	n.r.	...
Syrian Arab Republic	4.6	4.6	14.3	14.7	0.90	0.90	104	3.0	39.8
United Arab Emirates	...	...	...	...	0.64	0.67	n.r.	n.r.	...
Yemen	9.3	9.2	4.0	4.0	3.5	3.5	...	...	...

Source: FAO, *FAO Yearbook Production 1994*, vol. 48 (Rome, 1995), pp. 220-229. The Hashemite Kingdom of Jordan, *Statistical Yearbook 1994*, No. 45 (Amman, Department of Statistics, 1995), p. 153; (Central Bureau of Statistics, Syrian Arab Republic, *Statistical Abstract, 47th year* (1994), p. 159 and p. 161; *Industrial Commodity Statistics Yearbook* (United Nations Statistical Division, 1993), pp. 238-239 and p. 513.

... No data available or no production.
n.r. = not relevant.

In general terms, the existing wastewater treatment plants and wastewater collection system coverage and performance in the region are inadequate (108c). Quality controls of the effluent of wastewater treatment plants often disregard the end-use of treated wastewater: irrigation for agriculture purposes. The removal of pathogenic organisms, such as helminths, protozoa and bacteria, is crucial to avoid increased infection rates among the rural populations.

Domestic wastewaters in the arid areas of Western Asia are more concentrated for up to five times the pollution levels of sewage in Europe and the United States. This has serious consequences for the occurrence of water-born diseases such as ascariasis (roundworm), trichuriasis, hookworm and schistosomiasis. The incidence of infections is further aggravated by the use of effluent for irrigation purposes. In addition, the commonly used activated sludge-system is not efficient in removing pathogenic organisms.

Research and development activities in more advanced areas of enzyme and bioreactor technology are being carried out in Egypt, Jordan, Kuwait and the Syrian Arab Republic. The following sections will present the most important activities in some of these countries.

1. *Egypt*

Bioreactor technology in Egypt is utilized within many different sectors, ranging from biological nitrogen fixation applications in agriculture to the production of organic solvents and vaccines (see table 47). Bioreactor technology has a long history in Egypt as the first inoculum production of nitrogen fixing bacteria was achieved in 1939 at the ARC (5f). Bioreactor technology used for inoculum production of nitrogen fixing bacteria is the best mastered technology as five out of seven major research institutes working on bioreactor technology in Egypt are active in this field.

Commercial activities, however, are not reported in this field. For large-scale applications of bioreactor technology in industrial production (food, organic solvents, detergents, disease diagnosis kits) Egypt has to rely on the import of enzymes (mainly peroxidase and urease), the tools to carry out the digestion process (86b).

Concerning waste treatment, as of 1950 Egypt started to develop the capacity to produce methane (biogas) through the fermentation of rice straw. In 1986, around 50% of the available rice straw in Egypt (over 1 million tons) was utilized for composting and to a modest extent for methane production in small-scale rural based plants (5k).

Egypt hosts the Microbial Resources Centre (MIRCEN), established in 1978 at the Ain-Shams University Faculty of Agriculture: the aim is to provide bacterial and fungal cultures for free to research and industrial laboratories. The Centre strives to maintain a strain-pool covering all possible applications of micro-organisms.

In 1990 MIRCEN Cairo reported the distribution of 730 different strains to several research institutes and industrial corporations in Egypt (140). For carrying out its work, MIRCEN Cairo is dependent on occasional grants from foreign donors (such as USAID) to repair and purchase equipment for strain-pool maintenance (137). As with other agricultural research institutions, such as the Agricultural Research Centre (ARC) and other universities, the main experience and research of MIRCEN are geared to biological nitrogen fixation applications for agriculture.

The National Research Centre awards high priority to bioreactor technology to avoid overlap with the activities of the Agricultural Research Centre. The Engineering and Biotechnology Division spends approximately US$ 100,000, or 75% of its annual budget, on bioreactor technology (138). The department

of microbial biotechnology collaborates with a public commercial industry, the Egyptian Sugarcane Company, on the microbial digestion of sugarcane residues. Biotreatment of agro-industrial waste constitutes the major research activity of the division. In particular, research is focused on the bioconversion of solid wastes from a pulp and paper mill into single cell proteins (141). Funds for the research are provided by the Ministry of Science, collaborating government factories (e.g. Egyptian Sugarcane Co., El Nasr Co.) and foreign donors such as USAID (138). From 1990 till 1993, USAID sponsored a project through the ASRT on research for enzyme production for clinical and industrial applications in order to benefit the public El Nasr Company. USAID contributed US$ 173,000, and the Egyptian Government contributed US$ 212,570 to the project (86c).

TABLE 50. KEY EGYPTIAN INSTITUTIONS WORKING ON BIOREACTOR TECHNOLOGY IN EGYPT

Institute	Date of establishment	Main activities
1. Agricultural Research Centre	1930	Research: • Inoculum production for biological nitrogen fixation (1930) • Digestion of agricultural biomass for energy • Recycling of organic wastes for manure • Single Cell Protein production (enzymes) • Cyanobacteria production for biofertilization rice (1990)
2. NRC, Genetic Engineering and Biotechnology Division (138).	1960	Research: • Inoculum production for biological nitrogen fixation (1960) • Production of organic solvents • Single Cell Protein production (enzymes) • Digestion of agricultural/industrial waste (138)
3. MIRCEN-Cairo (137)	1978	Public Services: • Distribution of micro-organism strains to research and industrial institutes • Training courses on biofertilizers, utilization agro-industrial wastes, food microbiology etc. Research: • Digestion of agro-industrial wastes • Biofertilization • Microbiological contaminations in the food industry • Production of yeast
4. Organic Chemical Industries Company.	1986	Commercial Services: • Production of major organic solvents (ethanol, acetone, acetic acid), yeast and CO_2, using molasses and rice bran
5. Egyptian Society of Applied Microbiology	1989	Public Services: • Establish national network and a research and development plan on biological nitrogen fixation
6. Cairo University, Department of Microbiology, Soil Biotechnology Unit.	1990	Research: • Biological nitrogen fixation Public Services: • Exchange of micro-organism strains with other research institutes • Provide technical information on strains

TABLE 50. (*continued*)

Institute	Date of establishment	Main activities
7. University of Alexandria, Bioscience and Technology Department.	1990	Research: • Biotreatment of waste water • Digestion of agro-industrial wastes for energy • Improvement *B. subtilis* for detergents and food production
8. South Egypt Drug Industries, SEDICO	1993	Commercial Services: • Production of pharmaceuticals, nutrition products and cosmetics
9. El Nasr Company	*	Commercial Services: • Production of antibiotics and enzymes for industry, such as amylase (for desizing cotton) and proteases (for tanning leather)
10. Egyptian Organization for Biological Products and Vaccines (VACSERA).	*	Commercial Services: • Development of vaccines (against typhoid, tetanus, cholera, tuberculosis and schistosomiasis)
11. Animal Health Institute.	*	Research: • Development of livestock vaccines, growth hormones and antibiotics

Source: A. Sasson, *Biotechnologies in Developing Countries: Present and Future*, vol. 1 (Paris, UNESCO, 1993), p. 596 and pp. 603-604.

* No data available.

2. *Jordan*

In Jordan, research and development in enzyme and bioreactor technology are mainly being carried out in the veterinary industry, which will be reviewed in chapter VI.

Research on recycling and bioconversion of industrial and agricultural wastes is conducted at the Faculty of Agriculture of the University of Jordan. At present the HCST is financing a research project on the conversion of agricultural waste into single cell proteins (SCP) by using solid substrate fermentation technology. A pilot plant for the fermentation of animal waste to produce methane is being carried out by the Faculty of Engineering, Chemical Engineering Department, at the Jordan University farm in the Jordan Valley. The plant produces approximately 3.6 kwh of energy (144).

Research and development of bioreactor technology for food processing are confined to universities. The Faculty of Agriculture, University of Jordan, Amman, received financial support for training and equipment from the Canadian Agency for International Development (CIDA), totalling US$ 770,000 over five years (96a). This project aimed at developing the faculty research capacities in the applications of fermentation and enzyme technology, animal embryo transfer and semen processing, and plant tissue culture technology. The Nutrition and Food Technology Department received equipment, of a total value of US$ 23,264, for three 3-litre fermentors and a GLC (96b). This Department has succeeded in producing 15 international publications in international scientific journals since the finalization of the project (147). Before the project started in 1991, the Department only provided training to students. HCST provided a research

grant of 10,000 Jordanian dinars for two years starting in 1996 to investigate the production of broiler feed by using fermented olive pomace (147).

The University of Jordan, through its Faculty of Engineering, Chemical Engineering Department also initiated research on fermenting dairy-waste ("labaneh" whey) which contains 5% of lactose, into methane, using a 100-litre fermenter. Just after the second Arab Conference on Perspectives of Modern Biotechnologies, the Chemical Engineering Department obtained an order from a private company to perform research on the production of citric acid through fermentation of bacteria strains. However, after three months of research the company stopped its financial support. The Department also established strong contacts with the University of Hamburg, Germany, and the University of Nottingham, United Kingdom, in its research activities (144).

Jordan harbours a small detergent industry. Total export figures of detergents and soaps amounted to US$ 33.5 million in 1994 (9a,b). As can be seen from these export figures, the Jordanian detergent industry could well benefit from the local production of proteolytic enzymes.

E. Recommendations for ESCWA member countries

1. *Enzyme and bioreactor technology*

Enzyme and bioreactor technology are biotechnology research areas that are generally awarded less public attention than research areas such as recombinant DNA technology. This same trend is reflected in biotechnology research in the ESCWA region. Little research is conducted in this area and only in the cases of Egypt and the Syrian Arab Republic is research linked to large-scale industries. Typically, decisive factors for the economic viability of a bioreactor plant are not linked to the actual fermentation of a micro-organism but are decided on the basis of the costs of enlarging the operation and purification. There is thus a pressing need for allocation of resources for bioprocess-technology, which deals with large-scale applications of bioreactor technology.

As can be seen in tables 46 and 47, there is ample incentive, concerning investment opportunities, to explore related biotechnology research for food manufacturing and leather and detergent industries. The attempts of the Egyptian National Research Centre to collaborate with public companies are noteworthy, Research has targeted the production of medical and industrial enzymes using bacterial strains, which is a comparatively well established technology. This could well result in tangible results in the short term to be used by interested industries.

Most Egyptian research efforts in bioreactor technology, however, are confined to biofertilizer applications. The potential benefits of using *Rhizobium* and *Bradyrhizobium* based inoculant for improving quantity and quality of yield of leguminous crops have already been clearly demonstrated by international research. Prerequisites for such benefits are the availability of other nutrients and water for the crop. Only if these conditions are met can biofertilizers contribute to increasing the productivity of leguminous crops. Moreover, clear benefits of using inoculant have only been demonstrated for soybean. For most other crops, introduced inoculant could not compete with *Rhizobium* strains already present in the soil, in achieving subsequently higher yields. Only in separate cases where local *Rhizobium* strains are absent can biofertilizers contribute to crop yields and quality of pods. The use of inoculant for afforestation has been advised as beneficial in some cases, warranting more research in this area.

Syrian companies have already established some large-scale applications of bioreactor technology, as can be seen from the production figures in yeast and ethyl alcohol (table 46). Research on expanding production or the variety of products in these areas has not been reported. Especially within the

pharmaceutical industry there appear to be opportunities for the production of enzymes, both for medical applications as well as for industrial use (e.g. detergents, food-processing industry, paper industry). The pharmaceutical industry has considerable investment capital and raw materials such as enzymes are currently imported.

2. *Wastewater treatment*

As an alternative to more expensive and conventional wastewater treatment facilities, anaerobic digestion, notably the upflow anaerated sludge blanket reactor or waste stabilization ponds as suggested by the 1993 United Nations study (108b), may be better solutions for the specific conditions in this region.

The use of conventional aerobic wastewater treatment systems is basically dependent on priorities set by Governments and available financial resources. Considerable investments are required for wastewater treatment using aerated systems as activated sludge and trickling filters. Only the Gulf countries have sufficient financial resources to meet this challenge in the short term.

Alternative mechanisms, both in priority-setting and for technology development, may be explored to target the sustainable reuse of sewage. Research on developing alternative wastewater treatment technologies such as anaerobic systems may provide suitable alternatives in line with both sanitation and water reuse demands for irrigation. Anaerobic systems such as the upflow anaerobic sludge blanket reactor are, by their very nature (no aeration needed), cheaper to build and to maintain.

In the Middle East, population figures are still on the rise; this will mean a continuous increase of household wastewater disposal. This situation demands flexibility to expand wastewater treatment plants within short time-frames. Under these conditions, the UASB reactor would be an optimal solution as variation in size is much larger than with more conventional aerobic treatment facilities.

Surprisingly, the simplicity of wastewater treatment systems does not automatically imply a sound implementation of such technologies. Both Governments, municipalities, agencies responsible for wastewater treatment and farmers' interests should take into account implementation modalities where set policies meet the demands of target groups. The cities mainly want to get rid of their sewage. Farmers will want plentiful, cheap and "clean" water for irrigation of their crops. Somewhere along the line a compromise must be reached. "Clean" from the farmers' perspectives will mean clear water, whereas the UASB reactor will provide cheap murky brown water full of nutrients and clean in terms of biological contaminants. It is the most optimal solution for farmers.

Conclusions

1. It is Governments that must be relied on to promote the application of biofertilizers and wastewater treatment plants. In both cases, investments are not likely to result in direct returns on investment. In the case of biofertilizers, a market demand is not present and farmers will have to be educated to understand potential benefits. Only farmers who apply sound cropping practices are likely to benefit from biofertilizers. Farmers are therefore likely to rapidly lose interest in this market unless Governments provide additional incentives. Governments may be interested in biofertilizers since the use of properly inoculated leguminous crops can restore soil fertility over the long term.

2. In ESCWA region in particular, the benefits of sound biotechnology-based wastewater treatment methods are obvious. The reuse of treated sewage for irrigation purposes is a practice used to counter the increasing depletion of non-renewable water resources in the region. It is, however, not the availability of the technology that constitutes the crucial factor in its success. The formulation of agreements and the

coordination of collaboration between the partners of the technology transfer chain (e.g. municipalities - public research - sewage treatment plant - farmers) is needed to specify the available resources and needs for the technology. This will enable the right choice of a wastewater treatment system, from among the many model systems. Since most systems are well-researched, the technology is generic in nature and thus easily accessible.

3. It is clear that more direct commercial opportunities for exploring enzyme and bioreactor technology are to be found in the more conventional application areas such as food (including beverages), detergents and leather-manufacturing industries. These industries are well established in most of the ESCWA member countries. Local markets are large enough to warrant investment, and investors can expect short time-frames for returns on investments.

VI. BIOTECHNOLOGIES IN HUMAN HEALTH CARE

Introduction

Since the advent of the first transgenic product (e.g. insulin) for human use in 1982 (107a), medical biotechnologies have received increased attention from the public and the private sectors. Therefore, most commercial opportunities in biotechnology are in the field of human health.

Together, therapeutic and diagnostic biotechnology products accounted for almost 70% of total sales in the United States in 1994. This almost unilateral interest of the American private sector in medical biotechnologies has been prevalent for more than a decade (see table 50) Similar trends are also dominating in other industrialized countries. In Japan, out of 268 companies, 67% were involved in pursuing biotechnology research and development in pharmaceuticals and diagnostics, in 1987. In Western Europe 42% of companies were active in human health, in 1994 (168a).

TABLE 51. BIOTECHNOLOGY MARKET SEGMENTS OF SALES BY UNITED STATES COMPANIES

Economic sector	1986(%)	1994(%)[a/]
Human pharmaceuticals	20	42
Diagnostics	55	26
Equipment supply	15	15
Chemical, environmental and services	5	9
Agrobiotechnology	5	8

Sources: For 1986 data, see M. Avramovic, *An Affordable Development? Biotechnology, Economics and the Implications for the Third World* (London, Zed Books 1996), pp. 16-17; for 1994 data, see J. Bijman, *Strategies of US Biotechnology Companies*, Biotechnology and Development Monitor (Amsterdam, 1995), p. 14.

a/ Total 1994 sales amount to US$ 7.7 billion.

There are several reasons for the concentration of companies in medical biotechnology areas. The most important is that public spending for biomedical research is allocated more priority by national Governments. The first biotechnology products—insulin and monoclonal antibody-based diagnostics—were the direct results of this priority-setting, where basic public funded research paved the way for commercially viable technology development.

A second reason for the prevalence of biomedical research is that it is less restricted by environmental concerns and regulatory uncertainties. Although regulations for product development in medicine are stringent, the public system in most industrialized countries actively supports such R&D. Because medical biotechnologies may result in saving lives, ethical concerns about environmental issues are not as serious as they are in other areas, such as agriculture.

Commercial biotechnology research in medicine was also heavily favoured by the profitability of the pharmaceutical industry. This sector has consistently been among the most profitable sectors in the United

States economy, as well as those of other industrialized countries. In contrast in the 1980s, the period of most breakthroughs in biotechnology, the agriculture sector was going through a time of low profitability.

Worldwide sales in the pharmaceutical industry amounted to US$ 204 billion in 1994. Major markets are to be found in the United States., Europe and Japan (with US$ 64 billion, US$ 63 billion, and US$ 44 billion respectively), whereas the market of the Middle East and North Africa took a minor share of US$ 3 billion (9c). Globally, total R&D expenditure by the pharmaceutical sector amounted to US$ 30.1 billion in 1994. In addition, it was estimated that multinational companies invested between US$ 1.2 billion and US$ 7.5 billion on medical biotechnology R&D in 1995 (112).

This chapter highlights some of the most important areas of biomedical research. Developments in enzyme technology for therapeutic and diagnostic purposes will shape future expectations and the potential of this area. Monoclonal antibodies (MAbs) technology constitutes the most promising biotechnology area for medicine in the near future. As the first therapeutic applications for MAbs are taking place, an explosive market growth can be expected in the coming decade. The more established biomedical markets concerned with vaccines and antibiotics will also largely benefit from both DNA and protein engineering.

A. MEDICAL APPLICATIONS OF ENZYMES.

Enzymes are used for both diagnostic and therapeutic purposes in medicine. The use of enzymes as diagnostic or analytical tools is well established. Table 52 lists some of the major enzymes in use. Concerning diagnostic applications, enzymes mainly play a supportive role, as noted in chapter V. One of the biggest enzyme markets for medical diagnostics is centred around glucose oxidase. Glucose oxidase provides a sensitive specific test for the presence of glucose in blood and urine, for the diagnosis of diabetes.

TABLE 52. SOME ENZYMES OF IMPORTANCE IN MEDICAL APPLICATIONS*

ENZYME	Typical application
Trypsin	Anti-inflammation agent, wound cleaner.
Glucose oxidase	Glucose test in blood or urine.
Lysozyme	Recommended in treatment of certain ulcers, measles, multiple sclerosis, some skin diseases and postoperative infections (antibacterial agent).
Hyaluronidase (from beef testicles)	Hydrolyses polyhyaluronic acid, a relatively impermeable polymer found between human cells; administered to increase diffusion of co-injected compounds, e.g. antibiotics, adrenaline, heparin, and local anaesthetic in surgery and dentistry.
Streptokinase, Streptodornase	Anti-inflammatory agent.
Penicillinase	Removal of allergenic form of penicillin from allergic individuals.
Urokinase	Prevention and removal of blood clots.
Tissue Plasminogen Activator	Dissolution of blood clots.
Asparaginase	Anticancer agent.

Sources: J.E. Bailey and D.F. Ollis, *Biochemical Engineering Fundamentals*, 2nd edition, McGraw-Hill International Editions, p. 178.

* The enzymes are all of non-human origin.

A second well-established application area for biomedical enzymes is their use for curing serious and internal wounds. Proteolytic enzymes function both as digestive aids and cleansers of wounds. For internal wounds, digestive enzyme aids are suitably coated to protect the enzyme during its passage through the stomach, where the acid environment could cause protein denaturation. Injection of some foreign proteases

(pig trypsin differs from human trypsin) into human beings has been used to reduce tissue inflammation: the highly purified crystallizable form of the enzyme minimizes immune system response. The natural defences of live cells against protease attack are usually inactivated in dead cells. This convenient difference allows the application of solutions of proteases to virulent or oozing wounds: selective liquefaction of the dead tissue and cells is achieved, which facilitates wound drainage and thereby decreases the time needed for healing.

Proteolytic enzymes, especially trypsin, also serve to reduce inflammation and swelling. Swelling is usually associated with internal injuries and infections. Trypsin can help to dissolve blood clots, precipitating extracellular-proteins, or by locally activating other body defences which do the same thing. Some severe lung infections resulting in accumulation of viscous lung deposits have been reduced successfully or eliminated by proteolytic enzyme administration.

Therapeutic enzymes

As more than 1,000 diseases have been identified in which the absence or reduced activity of enzymes is the major cause of the disease, the use of enzymes as a therapeutic agent has increased considerably since the 1980s. Table 53 lists the major therapeutic enzymes applied in medicine. Enzyme production companies have been reluctant to invest time and money into specialty enzymes for medical use to treat diseases for which the patient market size is very small and the product is often required in high purity.

Recombinant DNA technology has increased the interest of pharmaceutical companies and has subsequently resulted in small specialty enzyme therapy markets. The recent application of recombinant DNase, which hydrolyses DNA, for alleviation of the severe lung congestion[1] in patients with cystic fibrosis is a recent example (118).

Genentech has produced the required DNase by recombinant methods using human DNase. With some 50,000 cystic fibrosis patients in the United States and Europe, it is anticipated that Genentech will recover US$ 100 million per annum from sales of the enzyme. By using recombinant DNA methods, the company has reduced its costs sufficiently to make the production of the enzyme commercially viable. Another promising future market for using genetic engineering is the production of human plasminogen activator.

TABLE 53. PRINCIPAL ENZYMES FOR MEDICAL THERAPY

Disease	Enzyme used for medical treatment
Cystic fibrosis	DNase; pancreatic digestion enzymes
Thrombosis	Tissue plasminogen activator; streptokinase
Osteoarthritis	superoxide dismutase
Cancer	• Nueraminidase (cell surface modification) • L-asparaginase; L-arginase (nutrient depletion) • Cytosine deaminase (prodrug activation)
Skin burns	Collagenase; bromelain
Liver disease	Cytochrome P-450 enzymes

Source: G.F. Bickerstaff, REVIEW-Impact of Genetic Technology on Enzyme Technology, *The Genetic Engineer and Biotechnologist*, vol. 15, No.1, Journals Oxford Ltd., p. 20.

[1] Lung congestion in cystic fibrosis patients may be caused by the disintegration of lung mucous whereby DNA is released from dead cells (human and bacterial). The DNA, being a highly charged macromolecule, acts as a cross-linking agent on the polysaccharide-based mucous and transforms the fluid mucous to that of a semi-solid gel. Hydrolysis of DNA by DNase returns the complex to a more fluid state that can be more easily expelled from the lungs.

Recombinant DNA techniques are increasing the potential applications of therapeutic enzymes in the near future, especially the market of therapeutic enzymes for the treatment of thrombosis, a disease which affects more than 10 million people in the United States alone, has prompted more than 100 companies to search for a cheap recombinant DNA protocol (113f).

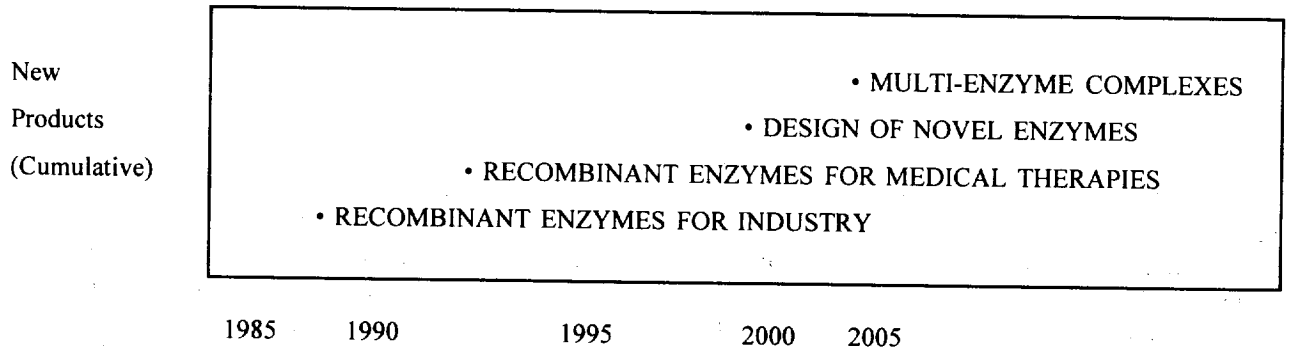
Thrombosis is caused by or associated with the formation of an abnormal blood clot (thrombus). Enzyme therapy has targeted the natural process of fibrinolysis and the products are designed to stimulate production of plasmin for natural hydrolysis of the fibrin clot. Human tissue plasminogen activator, which transforms plasminogen into plasmin, has been produced by recombinant DNA methods since 1988 by Genentech. Although the enzyme is more effective in dissolving the clot, the treatment is very expensive at US$ 2,000 per dose, in comparison with the commonly used bacterial streptokinase. Many companies are therefore looking for a cheaper alternative.

DNA recombinant technology also offers solutions for enhancement of purification. Specific chemical groups can be incorporated into enzymes, by genetic engineering, which can be used for subsequent one-step purification. Thus, an affinity tag is engineered onto the polypeptide chain in a suitable position on the recombinant enzyme, and the tag is designed to act as an affinity ligand in a chromatography extraction of the enzyme. This technique warrants some caution as the incorporated chemical groups may affect the final molecular structure of the enzyme, thereby negatively modifying the efficiency of the end-product. Removal of tags has been developed by incorporation of susceptible peptide bonds for cleavage by a highly specific protease.

The incorporation of new chemical groups into enzymes is part of protein engineering. Protein engineering is the alteration of the amino acid primary structure of a protein at the gene level with a view to enhancement of protein characteristics. Again, recombinant DNA techniques provide powerful tools for alteration of the protein structure of enzymes by site-directed mutagenesis to introduce amino acid substitutions and thereby alter fundamental enzyme characteristics. In time, this will lead to a class of new enzymes.

In the longer term, DNA recombinant technology can be used for the preparation of multi-enzyme complexes. At present, the vast majority of enzyme applications involve one-step reactions, most of which are hydrolytic. The future enzyme technology will focus developments on multi-enzyme complexes that convert a given substrate through as many as 10 reaction steps to produce a substantial biotransformation, for use in the synthesis of more intricate products. Multi-enzyme complexes would also have value in the analysis of multiple components, and to provide sequential hydrolysis of renewable polysaccharides. Figure XIII gives a time course estimate of developments in enzyme technology.

Figure XIII. Estimated time course for the introduction of major enzyme product groups



B. MONOCLONAL ANTIBODIES

Growing animal cells in culture is a method currently used for the manufacture of several products, including vaccines, the proteolytic enzyme urokinase, monoclonal antibodies, and interferons. Such processes also have substantial potential for production of other lymphokines (a group of proteins which regulate certain aspects of the immune system), other enzymes, growth factors, clotting factors, and hormones.

The advent of recombinant DNA technology introduces competition with animal cell cultivation for some of these products but also presents new possibilities for product manufacture using animal cell culture. On the one hand, the opportunity of expressing foreign proteins in micro-organisms means that microbial processes can now be used to manufacture these proteins in significant quantity. However, proteins naturally synthesized in animal cells are often subjected to several different types of post-translational modifications which are not accomplished in procaryotes. Furthermore, problems with proper protein folding and proteolytic attack make the expression of some eucaryotic proteins difficult in procaryotic hosts.

An example is insulin, used for treating diabetic patients, which is currently mass-produced by a construct of a human insulin encoding gene inserted into *Escherichia coli* bacteria. Recombinant human insulin was first produced in the late 1970s (107a). There have been some cases of human intolerance of this "human" insulin as compared with pig insulin, isolated from the pancreas of slaughtered animals.

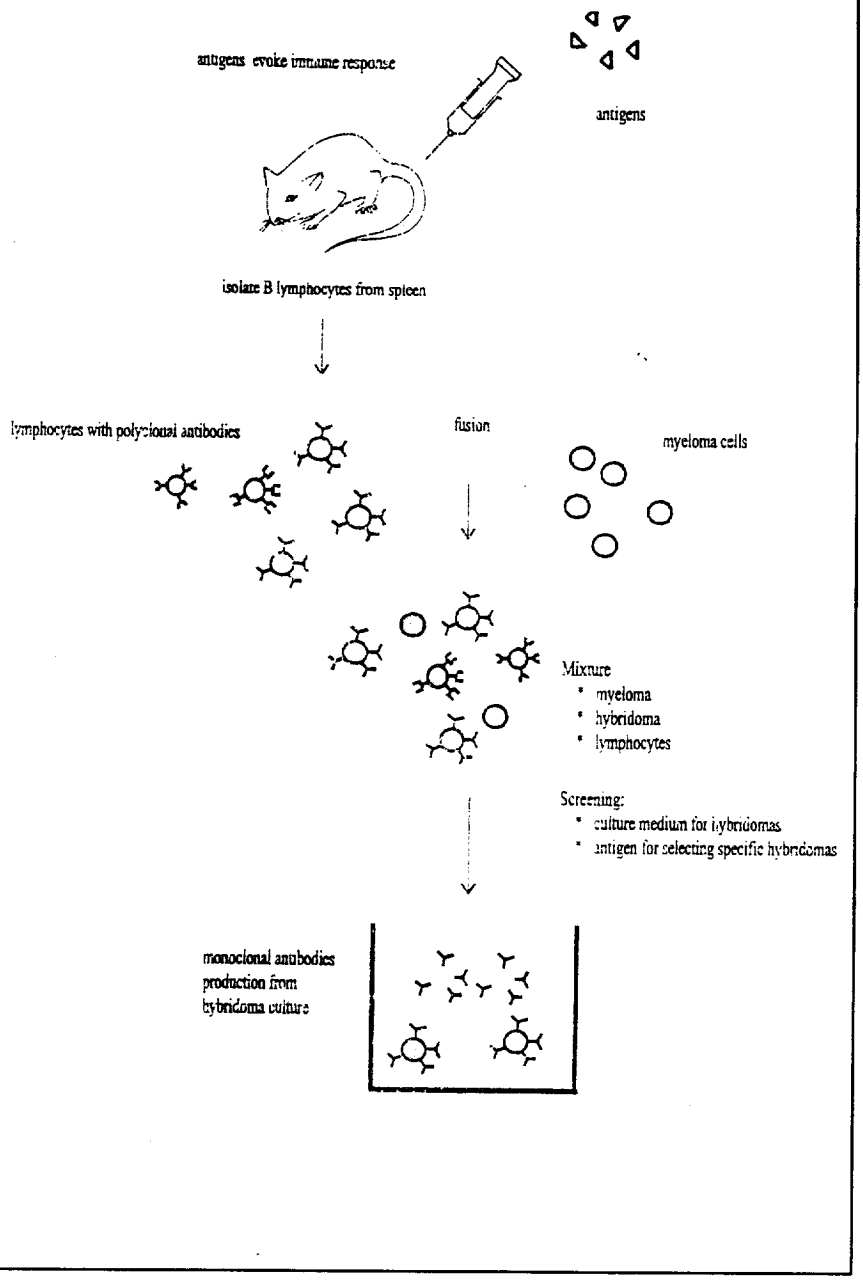
The single most important application of animal cell culture is the production of monoclonal antibodies or MAbs. Antibodies are most useful as a detecting agent for medical applications and research purposes. Normally antibodies are isolated out of the total blood of an animal which has been injected with an antigen of interest. The resulting antibodies are polyclonal, which implies that they not only detect the specific injected antigen, but also other antigens normally recognized by the immunological response of the vertebrate. Clearly the specificity, sensitivity, and reproducibility would be increased greatly if they could be carried out with *pure* antibodies.

To this end, the lymphocytic cells of a mouse were fused with myeloma cells in 1975. Myeloma cell lines are human cancer cell lines. By fusing the two cell lines, so-called hybridoma cell lines have been created which proliferate endlessly in culture, like cancer cells, but synthesize only one antibody (see figure XIV). The resultant antibodies are far more specific for a corresponding antigen, resulting in a higher level of reproducibility.

Although the technology for the production of monoclonal antibodies is reasonably well established, large-scale *in vitro* production is still difficult. Two basic bioreactor types used for large-scale production are the use of stirred tank batch reactors and hollow-fibre reactors. Productivity has been increased considerably, from 2 mg/week using *in vivo* hybridoma cultures to 2,000 mg/week using hollow-fibre reactors with a surface of 1.1 m^2 (163a). Although the use of stirred tank reactors results in lower yields (250 mg/week) compared with hollow-fibre reactors, the quality of produced immunoglobulins is considerably higher. In addition, the active fraction of hybridoma cells is smaller in hollow-fibre reactors, which is probably due to the formation of inactive aggregates, caused by the high density of cells in the hollow fibre.

There are several common features in the cultivation of plant and animal cells which complicate reactor design for their cultivation and for manufacture of their products. Many of the cell types of interest exist naturally as dense packings of similar cells. Such cell tissues in their native state are in contact with fluids of the organism of a specific composition and containing a variety of regulatory molecules which can strongly influence cellular function. Growth rates of the cells may naturally be very low. The challenge faced in submerged cultivation of these cells is to provide an acceptable environment for growth of the cells—many types of plant and animal cells when placed in culture do not grow at all. Once this obstacle has been

Figure XIV. Production of monoclonal antibodies



surmounted, the goal is to determine cultivation conditions that allow growth of cells to high densities in the shortest possible time, while retaining the metabolic capability of the cells to carry out the desired reactions.

Because of the expense of animal cell cultivation and because of slow growth rates which prolong experimental studies, quantitative characterization of the kinetic properties of animal cells is practically non-existent. Animal cell cultivation is generally expensive because of culture media requirements. Animal serum is one of the best media as it promotes cell replication and increases the cell resistance to mechanical damage. Serum, however is very expensive, with costs of up to $300 per litre (in 1986), accounting for 80% of the material cost when used at a level of 10% of the medium composition (106, p. 631). Alternatives to serum are hormonally defined media, such as those extracted from whole cow lymph. Ensuring sufficient oxygen supply to animal cell culture is another problem as animal cells have a low level of mechanical strength but require comparatively high levels of oxygen.

Source: C.K. Mathews and K.E. van Holde, *Biochemistry* (Redwood City [California], Benjamin/Cummings Publishing Co., Inc., 1990), p. 259.

There are basically two bioreactor types for animal culture, based on requirements of the used animal cell culture for attachment to a solid surface for growth. Cells from the bloodstream, lymph tissue, tumors, and many transformed cells can be adapted for growth in suspension culture. Other types of animal cells must be anchored to a compatible solid surface in order to grow. The design of reactors for larger scale cultivation of animal cells in suspension resembles microbial bioreactors.

Because of the high value of the product and the high operating expenses, a reactor as small as 10 litres may qualify as large scale. Of course, for cost reduction, large batch cultures are still to be preferred and reactors handling as much as 8,000 litres of culture fluid for interferon production were reported in 1985 (114a). A major obstacle that favours to the use of relatively small reactors for animal cell cultivation is the relatively slow growth rate of the cells and the more stringent requirements for sterile conditions. Animal cells are larger than microbial cells and do not have a cell wall, which makes them more vulnerable to mechanical shearing forces.

The use of recombinant DNA technology, whereby enhanced expression systems could be incorporated in cell lines, largely contributed to generating larger quantities of products. In addition, recombinant DNA techniques enable the production of completely novel monoclonal antibodies, consisting of a human region and a mouse region, which are less immunogenic than mouse antibodies. This is especially beneficial for therapeutic applications of MAbs.

The development of new monoclonal antibodies is considerably more expensive than the development of known MAbs. A 1983 estimate suggests that development of a new MAb supplied to *in vitro* kit manufacturers could cost between $3.5 million and $4 million over three years, with ultimate kit-production facilities costing 5-10 times as much (168e). Monoclonal antibodies first appeared in commercial use in the 1980s. Soon thereafter estimates for market shares indicated a big potential for the therapeutic use of MAbs (see table 54). In 1994, however, diagnostic MAbs still made up 75 percent of the estimated US$ 740 million US market for antibodies (123a).

Diagnostic uses of MAbs include hepatitis B, prostatic tumor, human venereal and hospital bacterial infections, pregnancy, rubella, and rabies, among others. MAb tests for blood type, pregnancy, drugs and infectious diseases are currently the most successful market components. Again, because of rapid developments in the field of therapeutic MAbs, this sector supposedly will encompass a market value of US$ 8 billion in US market sales by the year 2000 (123a). These predictions are made as more than 45 MAb therapeutics are presently in pre-clinical trials and should emerge on the market by that time. These MAb therapeutics include treatments for gram negative sepsis, cancer, autoimmune diseases (e.g. rheumatic arthritis), HIV and other virally transmitted diseases.

TABLE 54. MARKET SIZE DEVELOPMENT IN 1982 AND 1990 PROJECTIONS OF MONOCLONAL ANTIBODIES IN THE UNITED STATES

Application	Estimated market size (1981 dollars in millions)	
	1982	1990
Diagnostics		
1. In vitro kits	$5 to $6	$300 to $500
2. Immunohistochemical kits	Nil	$25
3. In vivo diagnostics	Nil	Small to $100
Therapeutics (Includes radiolabeled and toxin-labeled reagents)	Nil	$500 to $1,000
Other		
1. Research	Small	$10
2. Purification	Small	$10

Source: J.E. Bailey and D.F. Ollis, *Biochemical Engineering Fundamentals*, 2nd edition, McGraw-Hill International Editions, p. 827.

Because of the high costs for the development and use of therapeutic antibodies, non-industrialized countries will not have much access to the MAb market in the near future. At present, MAb drugs range in cost from US$ 2,000 to US$ 4,000 per dose (123a). When one considers that World Health Organization studies suggest that most developing countries spend US$ 5 per person for health care annually, the perspectives for the use of therapeutic monoclonal antibodies in non-industrialized countries are almost lacking (123a). Commercial prospects for non-industrialized countries for MAb therapeutics may change as new technologies, such as phage antibodies and combinatorial library screening methods, promise to develop MAb diagnostics and therapeutics at an unprecedented rate and cost. International research could also contribute to MAb research for tropical diseases, as an MAb drug for malaria is now in pre-clinical development.

In line with developments for enzymes for therapeutic purposes, antibodies are also redesigned for use as therapeutic agents, utilizing genetic engineering. The monoclonal antibodies developed according to the hybridoma principle cannot be used as a therapeutic agent since the human body still recognizes parts of the antibody as alien (i.e. parts of the mouse) and therefore mounts an immune response to destroy the injected antibodies.

In 1989 absolute human monoclonal antibodies were developed through the construction of so-called phage antibodies, human antibodies genetically engineered into *E. coli* bacteria, using phage as a vector (123b). In addition, this technique enables scientists to alter the design of an antibody by point mutations to increase the specificity of the antigen binding sites. This technology has recently opened up perspectives for the development of specific human monoclonal antibodies to be used as therapeutic agents. The first commercial releases are to be expected before the end of the millennium.

C. Vaccine technology

Vaccine technology is one of the most important biotechnologies in the area of health and medicine. Vaccines are generally based on culturing the causative organism, which is thereafter attenuated or rendered inactive. Since the technology involves working with potentially very dangerous micro-organisms, strict precautions against biohazards are a prerequisite. Advantages and disadvantages of the main two forms of conventional vaccines are indicated in table 55.

TABLE 55. COST-BENEFIT ANALYSIS OF THE TWO MAJOR CLASSES OF VACCINES

Costs-benefits	Live attenuated vaccines	Inactivated Vaccines
Development and production costs	Low	High
Dose requirements	Low, because micro-organisms will replicate.	High
Health risks (negative side-effects)	High, because organism may revert to wild-type infectiousness.	Low
Stability	Low, because live micro-organisms cannot tolerate high temperatures.	High

Source: B. Visser, New Roads to Vaccines, *Biotechnology and Development Monitor*, No. 17, pp. 6-7.

A number of vaccines, such as the vaccines immunizing against polio, measles, mumps and rubella, have routinely been produced through animal cell culture since 1954. The same technology is also used for

veterinary vaccines, such as foot-and-mouth virus vaccine. The production of this vaccine, produced in baby hamster kidney cells, was reported to be the largest bioreactor application of animal cell culture in 1990 (114a).

Conventional vaccines have gradually been replaced by new types of vaccines which are based on recombinant DNA techniques, as is the case with Hepatitis B. For instance, DNA manipulation techniques allow for site-directed mutations and/or deletions in one or more of the virulence determining genes that do not intervene with the pathogen's immunogenicity. The design of such attenuated strains results in vaccines that are both efficacious and safe.

A second type of vaccine is based on small subunits (e.g. protein, carbohydrates on the cell surface) of the pathogenic organisms, called antigens, which will trigger an immunological response. Using genetic engineering, antigens can now be produced by a different organism than the original pathogenic organism, whereby the final vaccine will be inherently more safe than an inactivated vaccine.

A third class of new vaccines involves the use of a live recombinant carrier. It combines the properties of a live attenuated vaccine and a subunit vaccine. For this purpose the genetic information encoding an antigenic determinant is incorporated into the genome of a host organism that is used as a live vaccine and from which non-essential genes, e.g. those encoding specific virulence factors causing disease, may have been removed. The latter class of vaccines uses vaccinia and other pox viruses, herpes viruses and adenoviruses as carriers. The main advantages of these type of vaccines include the potentially high level of protection, its physical and genetic stability, and its potential to be used as a multivalent vector against several pathogens.

Very recently, that is, since the beginning of 1996, an even more spectacular class of vaccines has been subject to human trials against AIDS (acquired immune deficiency syndrome) and cancer. The vaccine consists of naked DNA that encodes for antigens of the causative disease agent. Preliminary findings suggest that such vaccines may be more effective than injecting the antigens directly (115).

New vaccines might form a more effective strategy for the control of more complicated, higher-organized, uni-cellular pathogens, such as the causative agents of malaria and leishmaniasis. Leishmaniasis is a prevalent disease in Western Asia and is endemic in the Jordan Valley. It has proved difficult to control these pathoges, which may change it appearance during the various infectious stages, by using conventional vaccines, owing to their great adaptability and variability.

The vaccine developed against malaria by a Colombian research institute only provides 40% protection, but is still unique in that it is the first synthetic vaccine, that has shown some efficacy against a parasitic disease (128).

Another new class of vaccines, which is currently in the clinical test stage is made up of anti-fertility vaccines. The anti-fertility vaccines that are being tested are based on the mode of action of conventional vaccines against diseases. They are based on the stimulation of the immune system but—unlike anti-disease vaccines that target foreign substances,—anti-fertility vaccines evoke antibodies against the body's own substances (e.g. hormones).

One of the main technical problems is the stability of the vaccinia that is used as the carrier. Another problem is that since the vaccine stimulates attack on the body's own tissue, this might stimulate the incidence of auto-immune diseases. Moreover, the technology in itself is controversial as it facilitates the control of fertility for demographic purposes instead of enabling people to gain control of their own fertility (127a).

The production of vaccines for immunization programmes is one of the most promising biotechnology fields for developing countries from an economic and human welfare point of view. Immunization using vaccines is the most cost-effective way of disease prevention. It is estimated that WHO immunization programmes prevent the deaths of 2.2 million children annually (122a).

Surprisingly, there has been only limited interest shown by the private sector in the development of vaccines for the industrialized world, and virtually no interest in vaccines for non-industrialized countries. The reasons are simple. Vaccines account for only 1% of the profits of the pharmaceutical industry, and a greater percentage of their liability. One of the most important vaccines licensed in the past 10 years is a subunit vaccine against Haemophilus influenza b, the main cause of meningitis and mental retardation in young children around the world. Development costs for the company involved have been between US$ 8 million and US$ 12 million (122b). Generally it will cost between US$ 20 and US$ 30 million and require 7-10 years of development and clinical testing to perfect vaccines available to the public (130).

For live vaccine vectors, such developments costs would be even higher, and this explains the disinterest of pharmaceutical companies in industrialized countries in research and manufacture of low-priced vaccines. Worldwide, there are only a few (mostly private) institutions that can bear the total costs involved in all aspects of the development of new vaccines. Both in the public and private sectors, the development and production of vaccines is only regarded as economically viable, if a significant market of users can be reached. Estimates vary between 25 million and 100 million potential users, depending on the specific disease and the required technical approach (129). As a consequence, in many cases the development and marketing of a new vaccine needs to take place in an international or regional, rather than a national framework.

It is a hopeful sign, however, that some non-industrialized countries (e.g. Brazil, Colombia, Cuba, India and Mexico) have expanded their own capacities for the development of vaccines, with encouraging results. Vaccines developed in non-industrialized countries include vaccines against leprosy (developed in Venezuela and India), leishmaniasis (Venezuela and Brazil), dengue haemorrhagic fever (Thailand), and malaria (Colombia). The research institute in Colombia working on the malaria vaccine turned down the US$ 8 million offer of a large pharmaceutical company and instead handed over all rights of the vaccine to WHO. The Colombian Government has offered to build a production facility (128).

Under the auspices of WHO, fertility-regulating vaccines have been developed; they are currently the subject of clinical trials in several countries. Tests are being conducted in India, Australia, Brazil, Chile, the Dominican Republic, Finland and Sweden. The first clinical trials with anti-fertility vaccines began in the 1970s. At present almost 10% of the US$ 57 million spent annually on overall contraceptive research is devoted to anti-fertility vaccines (127b).

D. ANTIBIOTICS

Antibiotics are classified as secondary metabolites, compounds not essential to growth but providing a supplementary role for the survival of the organism. Antibiotics consist of a very diverse group of low molecular mass organic compounds, which specifically kill different classes of micro-organisms. Originally antibiotics were of biological origin, produced by spore-forming soil micro-organisms during or just prior to sporulation, as a defence mechanism against potential competitors. These secondary metabolites function in general by blocking one or more enzyme reactions in the affected cell.

Antibiotics are part of a larger group of antibacterial medicines on which development started in the 1930s. Some 5,000 antibiotics are known, which form the basis of large commercial industry (116a). Nearly

100 antibiotics are produced commercially via fermentation. To increase the range of antibiotic agents, natural occurring antibiotics have been chemically modified to yield semisynthetic antibiotics.

Semisynthetic antibiotics are constructed from the core molecule of a natural antibiotic to which side-groups are added or removed using organic synthetic chemistry. Increasingly, antibiotics are fully synthesized, reducing the future potential for the fermentation process of producing antibiotics. Fully synthetic products include chloramphenicol, pyrrolnitrin and efrotomycin. Innovations in antibacterial medicine have been classified into four technological trajectories indicated in table 56.

TABLE 56. THE FOUR TECHNOLOGICAL TRAJECTORIES OF ANTIBACTERIAL MEDICINES AND THE YEAR OF THEIR COMMERCIAL RELEASE

Trajectory	First Product	Year
Sulphonamides	Prontosil	1935
Natural Products (Antibiotics)	Penicillin	1942
Semisynthetics	Tetracycline	1953
Synthetics	Nalidixic acid	1963

Source: B. Achilladelis, *The Dynamics of Technological Innovation: The Sector of Antibacterial Medicines*, Research Policy 22, North-Holland, 1993, (Center for Economic Policy Research, Stanford University) p. 281.

Total 1980 estimated world annual production and sales of antibiotics amounted to approximately 10 tons in volume and US$ 4 billion in profits (106n). Since the 1930s antibiotics have saved millions of lives and formed the core operation of many pharmaceutical companies. The need for more and new antibiotics changed the pharmaceutical industry into a research-intensive industry. This was mainly due to the great commercial success of the antibiotics, and the potential they offered for imitation and improvement.

Commercial biotechnological production of the first antibiotic, penicillin, was started in 1942 by the pharmaceutical companies Pfizer and Merck (165b). The road from discovery to final production took 14 years, owing to conditions listed below that are often associated with innovation failures:

- The interests of Alexander Fleming (who developed penicillin) were primarily academic.
- St. Mary's Hospital, his employer, did not possess the essential expertise in organic chemistry.
- Commercial and industrial interest and expertise were lacking.
- No Business innovators, third party entrepreneurs or research-intensive companies were involved.

It was only through the intervention of the British and United States Governments, which brought the necessary actors together in 1941, that the production of penicillin became commercially viable. Based on the experience in developing and marketing penicillin, the British Government established the National Research and Development Corporation (NRDC), the aim of which was to provide funding for the development of British inventions that the private sector was reluctant to adopt. This resulted in the development of the cephalosporines that were commercially released in 1955 by Glaxo. Similarly the United States Government realized the importance of support for academic research and established the National Science Foundation. American pharmaceutical companies realized the potential of British academic research in antibiotics and acquired British R&D laboratories in the early 1950s. This move proved successful as many of the new American-produced antibiotics were discovered in these British laboratories.

As part of the penicillin project in the 1940s, the United States Office for Scientific Research and Development provided many incentives to pharmaceutical companies to explore the new market. Up to the end of the Second World War, all penicillin output was bought by the United States Government, which paid cost plus prices of about $ 20 a dose. The first producers made considerable profits up to 1946, when the United States Government imposed a ban on commercial sales, sold the plants at half price to the companies operating them, and offered the licence to any company that could demonstrate that it possessed the skills to become a producer.

Surprisingly, many non pharmaceutical companies jumped at this opportunity. Over time, these firms (e.g. Bristol Meyers and Upjohn) became multinational pharmaceutical companies. The United States Government's policies for the promotion of the antibacterial medical industries encouraged through financial and technical means the diffusion of the technology within national boundaries, while excluding foreign competitors. The policies set by the United States Office for Scientific Research and Development undoubtedly contributed to the emergence of the American pharmaceutical industry as the world leader in this field (165c).

From 1946 to 1949, worldwide price levels for penicillin dropped from the original US$ 20 per dose to US$ 0.10 per dose (165c), owing to intensive competition promoted by the incentives of the United States Government. Because of new patent and trademark legislation covering natural products in the United States and the advent of a new market for antibiotics—animal farming—the pharmaceutical industry pursued research and development activities in this area. The first antibiotic to be developed by modifying a natural antibiotic was tetracycline (1953), which gave rise to a new generation of antibiotics, the so-called semisynthetic antibiotics. These developments were partly triggered by the following shortcomings of natural penicillin:

- Quick secretion;
- Need of parenteral administration (painful injection);
- Reduced effectiveness of enzymes produced by Gram-negative bacteria;
- Increasing resistance of Gram-positive bacteria;
- Adverse side-effects on patients.

During the decade that followed, 37 antibacterial medicines were introduced, of which 11 were natural products and 17 were semisynthetic antibiotics based on natural penicillins and tetracyclines. In the 1970s there was a general decline in the introduction of new antibiotics which was attributed to the severity of regulatory procedures. By the end of the 1980s, the semisynthetic cephalosporins were introduced on the market, giving rise to a new class of wide spectrum antibiotics. Of the 70 new products introduced in the 1980s, 29 were semisynthetic cephalosporins, 8 were semisynthetic penicillins and 13 were natural products (165d).

The large-scale production of natural antibiotics usually takes place in conventional continuous-flow stirred tank (CSTR) reactors (116b). The final bioreactor size for the production of antibiotics is in the range of 10,000 to 300,000 litres. This type of reactor was reviewed in chapter V of this study.

The most likely opportunities for developing new antibiotics are mainly in synthetic organic chemistry, with the introduction of the quinones in the 1990s. For mid-term planning, technical opportunities for pharmaceutical industries will remain a multidisciplinary exercise, requiring microbiologists to identify promising new micro-organism strains for natural antibiotics and to test antibiotics. Bioprocess technologists are required for upscaling, fermentation, isolation and purifaction processes of bioreactor technology. Organic chemists are required for analytical isolation and purification of active antibiotic compounds. Synthetic organic chemists are needed for modifying or fabricating entirely new antibiotics. The use of genetic

engineering of micro-organisms for the production of antibiotics includes molecular biologists in this field of research. Whatever the technical opportunities may be, market considerations will always be decisive for the launch of innovations in a sector which has reached maturity and which needs and time-consuming and expensive marketing campaigns.

The expiration of patents of successful antibiotics is market force as it leads to the entry into the market of generic drug manufacturers and sharpens price competition. Thus, innovative companies create a flow of new and comparable products, to replacing the current product when patent production expires. The substantial growth in value compared with volume of sales of antibiotics in the 1980s was partially due to this type of replacement, as the new medicines are sold at considerable higher prices than their predecessors. This strategy maintains a pressure on leading companies to invest heavily in research and development of new products.

Achilladelis (165d) indicates a strong relation between so-called radical innovations in antibacterial medicines and market success. A total of 27 out of 42, or 64%, of radical innovations were directly linked to big market successes between 1934 and 1990. A "radical innovation" describes innovations based on different scientific principles, technology or materials which have replaced or competed successfully with existing products and processes, and gave rise to a bulk of incremental innovations.

The incremental innovation, or innovations based on slight adjustments of a radical innovation, however, are strongly associated with market failures (i.e. 79% of a total of 130 between 1934 and 1990) (165d). In other words, the originality of the antibiotics industry's R&D is crucial to ensuring large economic success and a leading market position. A corporate technology tradition is, however, not a prerequisite to competing in the antibiotics market. Innovations in antibacterial medicines are highly concentrated among a few companies: the top five companies account for 35% of new products.

However, many smaller pharmaceutical companies conduct modest or no research, with no or very few innovations, and still maintain a considerable level of market sales. Overall, Governments—and the public sector in general—play a critical role in pharmaceuticals as regulators, customers and supporters of R&D projects that industry considers too risky to undertake, as illustrated by the above example of the development of penicillin. Governments, therefore, have an important role in developing the domestic expertise of the pharmaceutical industry.

The case of penicillin and the subsequent emergence of an entire industry clearly illustrates that a scientific discovery in itself is not sufficient to guarantee commercial success. It requires the development of the associated technology for the large-scale production that determines the economic viability of new scientific breakthroughs.

Vitamins and steroids

Of the microbially synthesized vitamins, only B_{12} and B_2 are produced microbially at present, and the latter has been progressively displaced by synthetic routes. The biotechnological production processes of vitamin B_{12} that are economically feasible depend either on Propionibacterium or Pseudomonas. The Pseudomonas process seems to be of greater economic importance. In the early 1980s, world production of vitamin B_{12} was roughly ten tons (106o), of which the pharmaceutical industries consumed about two thirds and the balance went to animal food production.

Although vitamins are increasingly produced by pure chemical synthesis, the most efficient industrial means of production of L-ascorbic acid, or vitamin C, is a semisynthetic route utilizing the fermentation of Acetobacter suboxydans for the intermediate transformation of d-sorbitol into l-sorbose. Subsequently this

compound is chemically converted in several steps to l-ascorbic acid. The method is very efficient and requires only 2-4 kg of glucose to produce 1 kg of vitamin C (164a).

The synthetic capacity of the various micro-organisms makes it theoretically possible to produce all vitamins by way of fermentation. However, synthesis of vitamins according to the methods of organic chemistry— with the exception of vitamin B_{12}—still maintains a dominant position in industrial production. The use of genetic engineering of micro-organisms to improve the production of vitamins will change this situation in the near future.

The use of biotransformation, using microbes or enzymes for the biochemical conversion of steroids, reduced costs of the production of steroids considerably. For instance, the production of cortisone out of progesterone reduced costs from US$ 200/g in 1949 to US$ 1/g in 1979 (106o). Applications for natural and derivatized steroids include therapeutic uses (estrogens, progesterone, and androgens); contraceptives (derivatized estrogen and progesterone); sedatives; antitumour therapy; veterinary products; and anti-inflammatories for skin diseases and arthritis (cortisone).

E. Regional developments

1. *Egypt*

In Egypt, biotechnologies' applications in the field of health and medicine consist of the commercial production of vaccines and antibiotics using bioreactor technology.

Advanced molecular biology research in medicine is conducted by the Faculty of Medicine, Cairo University. The Faculty obtained two grants from the Science and Technology Cooperation (STC) project, administered by ASRT and funded by USAID. These subprojects were planned for the period 1992 to 1996. Both projects were extended to June 1996 owing to delays due to administrative procedures (117).

The first grant consisted of US$ 194,554, with a contribution in kind from the Egyptian Government in the amount of US$ 74,753 (86d). The subproject aims to apply modern techniques in molecular biology (PCR) for the detection of minimal residual disease in chronic myeloid leukaemia patients who have undergone bone marrow transplants. In cases where disease is detected through PCR, savings on additional operations and post-operative care could amount to US$ 22,059 per patient (86d). Since the cost of an early diagnosis of chronic myeloid leukaemia amounts to only US$ 294 (86d). The subproject also identified new types of chromosomal disorders.

The other STC grant consisted of US$ 170,082, donated by USAID, and US$ 80,153 (in kind), donated by the Egyptian Government, to the Faculty of Medicine at Cairo University (86e). This subproject aims to apply the PCR technique for the detection of human papilloma virus in biological tissues and in smears of cervico-vaginal cells. Early diagnosis of cervical cancer would reduce costs and enhance cure rates considerably. The cure rate would increase from 40% to 95% and the costs for treatment would diminish from US$ 2,941 to US$ 294 per patient (86e). The STC could not report the implementation of the PCR technique for the diagnosis of chronic myeloid leukaemia or cervical cancer by the anticipated end-user, the National Cancer Institute.

The Department of Molecular Genetics, National Research Center, has been active in research for establishing diagnostic tools in medicine since 1987 (see table 57). At present the department is working on the production of RNA-PCR kits for the diagnosis of viruses.

TABLE 57. RESEARCH PROJECTS CONDUCTED BY THE DEPARTMENT OF MOLECULAR GENETICS, NATIONAL RESEARCH CENTER (EGYPT)

Year	Research	Sponsor
1987	Prenatal detection of homozygous and heterozygous carriers for beta thalassemia in Egypt	*
1990-92	Oncogene activation of bilharzial bladder cancer in Egyptian patients	USAID and Ministry of Health.
1992-93	Study to assess the mutagenicity of Schistosoma eggs.	USAID and Ministry of Health.
1994-96	Identification of tumor promotor(s) in Schistosoma eggs	USAID and Ministry of Health.
1995-96	Production of HCV-RNA PCR Diagnostic Kit	*

Source: N.A.M. Saleh, Vice-President for Research, National Research Centre, Cairo, Egypt. Personal communication, 1995.

* No data available.

2. *Jordan*

In Jordan, research and development in enzyme and bioreactor technology are mainly taking place in the veterinary industry.

The best established company using bioreactor technology in Jordan is the Jordan Center for Veterinary Vaccines (JCVV). JCVV was established by the Government of Jordan and the German technical cooperation organisation GTZ (Gesellschaft für Technische Zusammenarbeit) in May 1988. The capacities of JCVV for the production of vaccines for veterinary purposes are well established and GTZ has invested large sums of money and manpower in JCVV since 1984. Up to September 1996, GTZ will have invested a total of 9.2 million deutsche mark (approximately US$ 6 million) and the Jordan Government will have invested a total of 3.25 million Jordanian dinars (JD) (approximately US$ 4.6 million) (119).

The Center is capable of mass production of various viral and bacterial vaccines for veterinary purposes. The quality of production is reported to be of an international standard. Facilities are also suitable for the production of human vaccines. At present the total production capacity is underutilized as only 1% of the total output capacity is utilized. The total production capacity could cover a major part of the total regional demand for veterinary vaccines. A major impediment at present is the low productivity level. Therefore, unit prices cannot as yet compete with internationally produced vaccines.

If the production would double, JCVV would be able to compete on the international market. The funding support of GTZ on behalf of JCVV was scheduled to end in September 1996. Commercial production of veterinary sera and vaccines will only be feasible by involving private Jordanian enterprises to ensure investments in the long run. This has involved major negotiations between the Jordan Government (Ministry of Finance), which until recently was the main shareholder (40%), GTZ and private companies. Through intense negotiation efforts by GTZ, JCVV was privatized at the end of 1996. The vaccine production plant adheres to ISO 9000 standards which can ensure access to export markets (142).

Many pharmaceutical companies in Jordan are marketing drugs produced with biotechnology input. Few, however, are involved in biotechnology research and development or in the production of

biotechnological drugs under licence. One such company is ARCOMEX, founded in 1991 and specialized in the production and marketing of medical test kits. The company received a grant of JD 6,000 (US$ 8,450) in 1995 from HCST to do research on replicating toxoplasma bacteria (a major cause of miscarriages in humans) in rats in order to isolate antigens. From those antigens ARCOMEX aims to isolate specific antigenic determinants which can then be used for the production of vaccines. ARCOMEX is also trying to operate a 200-litre fermentor to cultivate Aspergillus niger for producing glucose-oxidase, an enzyme commonly used in test kits for diabetics. Currently, they have to import the enzyme glucose-oxidase at high costs (143).

The Jordanian pharmaceutical companies have the potential to produce biotechnology-related products. Like the pharmaceutical sector in the Syrian Arab Republic, Jordan has witnessed a boom over the last decade. Export figures of Jordanian medicaments increased from US$ 4.5 million in 1980 to almost US$ 129 million in 1994 (9b). The Jordan market for medicaments amounted to US$ 92.7 million in 1994, of which US$ 34.4 million were sales from local manufacturers. Local pharmaceutical companies have witnessed an increase of export figures, but domestic sales have decreased. Major export markets for Jordanian medicines are Saudi Arabia, Iraq, and East European countries.

For raw materials, which include biotechnology products such as organic compounds, penicillins, streptomycins, and other antibiotics, the Jordanian pharmaceutical industry has to rely on imports. In 1993 the total demand for raw materials, including biotechnology-affiliated product groups imported for the Jordanian pharmaceutical industry, reached a total of US$ 35.7 million. Table 58 indicates net imports of raw materials for the pharmaceutical industry in Jordan.

One Jordan based pharmaceutical company (Al Hikma) is producing cephalosporine intermediates. The company imports the raw material for which fermentation technology is used (150). The Jordanian Pharmaceutical Manufacturing Company is contemplating the production of biotechnology related diagnostic agents (149).

TABLE 58. NET IMPORTS OF RAW MATERIALS FOR THE PHARMACEUTICAL INDUSTRY IN JORDAN, COMPRISING BIOTECHNOLOGY DERIVED PRODUCTS
(Thousands of US dollars)

Compound	1989	1990	1991	1992	1993
Penicillins	363	20	0	0	0
Streptomycins	427	65	21	0	0
Other Antibiotics	15,157	9,791	10,645	14,936	29,586
Other Organic Compounds	956	7,848	10,219	14,241	6,147
TOTAL	16,903	17,724	20,885	29,177	35,733

Source: Industrial Development Bank, *personal communication, 1995.*

3. *The Syrian Arab Republic*

Reports on the considerable size of the pharmaceutical industry in the Syrian Arab Republic in which a total of 44 private companies and 2 public companies are active with total market sales of approximately US$ 120 million (145), indicate the potential of biotechnological product groups such as hormones, antibodies and vaccines. However, except for some microbial contamination control, there are no reports on using

biotechnology in the production of pharmaceuticals. One plant in Aleppo produces intravenous vaccines; another plant in Damascus plans to produce hormones and other plants report the production of antibiotics. The raw materials of these product groups, for which biotechnology is a prerequisite, are all imported.

Most local producers claim that the Syrian market is too small to warrant the production of raw materials. They will, therefore, argue that the country should export such raw materials in order to expand market opportunities. Such opportunities are further limited because of a lack of trade agreements with surrounding countries concerning such export and, more important, the affiliated international pharmaceutical companies do not allow their licence holders to produce the raw materials themselves.

In the field of veterinary vaccines, a small plant was set up as a joint project between GTZ and the Ministry of Agriculture. Production covers 70% to 80% of the total Syrian market (145).

F. POLICY RECOMMENDATIONS

In general, the pharmaceutical sector in Western Asia faces several restrictions on investing in biotechnological research. In addition, pharmaceutical companies in the region are not used to conducting research. Another problem is that, by ceasing to import enzymes from international pharmaceutical companies and starting their own production lines, pharmaceutical companies based in the ESCWA region risk losing their licence to produce, from the international companies.

International companies restrict their licence holders with regard to exporting medicines to other countries. Local markets are too small to warrant the production of biopharmaceuticals such as vaccines and antibiotics. This is clearly demonstrated by the Jordan Center for Veterinary Vaccines (JCVV), whose plant production capacity could well cover a major part of the regional demand. However, since the company has not established links with regionally operating marketing agents for pharmaceuticals, this market is inaccessible to JCVV.

The pharmaceutical industry in ESCWA member countries is a sector in which both private industries, Governments and consumers have common interests. Local private industries would strengthen their corporate standing by investing in biotechnology research and development, producing their own raw materials (e.g. enzymes) and gaining access to the lucrative monoclonal antibody market. Governments would benefit from many factors, including a decreased health bill (through import substitution), increased employment and training opportunities, and a strengthening of the country's export position. Consumers would benefit from lower prices and more access to advanced medicine.

However, in order for the pharmaceutical sector in ESCWA member countries to strengthen their position in research and development, regional coordination is needed, and therefore standardization of regulations for production and trade. Although intellectual property rights are high on the agenda of international pharmaceutical companies, this does not necessarily require local pharmaceutical companies to also allocate prime importance to this issue. By first starting research and development activities in established biotechnologies such as conventional production of enzymes (e.g. glucose oxidase by *Aspergillus niger*), intellectual property rights would not be an issue.

Moreover, since the patents of many major drugs have expired in recent years, the United States market for generics is expected to double between 1994 and the year 2000 (9d). It should be noted, however, that since most industrialized countries are trying to contain costs of health care, this decreases the profit margins of the pharmaceutical industry. Major multinational companies have adopted four different strategies to deal with these new trends (see table 59).

TABLE 59. MAIN STRATEGIES UTILIZED BY MULTINATIONAL PHARMACEUTICAL COMPANIES TO RESPOND TO NEW TRENDS IN COMPETITION

Strategy	Elements
Vertical integration	• Coupling of production to drug distribution and providing information on drug usage to gain more market-share. • Couple the sale of pharmaceutical supplies with the provision of health care services. • Integration to be realized through acquisitions or alliances.
Horizontal integration	• Increase the size of core processes (e.g. selling, marketing, managed care presence, administrative activities). • Integration to be realized through acquisitions or alliances.
Drug innovation	• Focus on achieving breakthroughs in research for specific diseases.
Disease management	• Address all aspects of a disease in an integrated programme that reduces health care spending. • Programmes are often targeted at improving drug compliance and reducing side effects.

Source: Industrial Development Bank, *personal communication, 1995.*

Most large and well-established pharmaceutical companies are increasing pressure on developing countries to establish more stringent patent laws, as noted in chapter IV. The stand of such corporations is mainly motivated by the increase in global competition because of improved communication technologies. Small pharmaceutical companies based in developing countries stand to lose on two fronts. First, they are and will be excluded from foreign markets by licence agreements with the multinational companies. The new companies need such agreements in order to obtain raw materials and produce up-to-date drugs (although under licence). Secondly, they will lose domestic market size as their product development cannot compete efficiently with patent-protected new drugs from large multinational companies.

With regard to the potential benefits of biotechnology for the pharmaceutical sector in Western Asia, the following observations have been noted. The pharmaceutical industry in Western Asia is completely based on the production of generic medicines. This market is rapidly being saturated as new domestic production plants are constructed at a higher pace than the growth of pharmaceutical market sales. One recommendation is to award more government support to the educational system in areas of interest to the pharmaceutical sector.

Such a commitment by the Government can only be expected if the private sector is also willing to invest resources in research and development. In other words, a dual commitment is required. As basic education (i.e. high school) is already of a good standard in the region, emphasis should be on higher education. The recommendations for integrating public research and development with pharmaceutical companies are as follows:

(a) Establish a mutual government-private industry revolving fund for research and development, with a steering committee to monitor expenditure of the revolving fund for research and development on the following:

(i) Manufacturing pharmaceutical products, covering the practical aspects of improving production (e.g. large-scale process technology);

(ii) Identifying new products (e.g. raw materials, intermediates, additives, diagnostics, therapeutics);

(iii) Clinical trials at hospitals, to evaluate the effectiveness of treatments;

(iv) Socio-economic aspects of market development and study of market prospectives of products.

Both the Government as well as private companies should be represented in the steering committee of the revolving fund. The amount of decision-making power of the steering committee should be directly related to the financial contribution of each participant. The steering committee should be composed of representatives of Government and private companies, reflecting the contribution of financial resources to the revolving fund.

(b) Increase basic research expenditure at universities through government and private sector intervention:

(i) Increase in government expenditure for practical research training (e.g. equipment, chemicals, supervision);

(ii) Universities should give incentives to faculty members to increase their research activities. The following suggestions may allow for a gradual increase in R&D activities at universities:

a. Increase salaries of staff according to research experience;
b. Streamline supply of chemicals and equipment;
c. Encourage staff to publish in reputable journals, and encourage additional research and/or travel through additional funds;
d. Set standards and regulations for a favourable division of time between educational and research tasks;
e. Allocate a budget for research collaboration with universities from industrialized countries.

(iii) Once a sound R&D base has been established, faculty members should be authorized to work full time on research proposals of private industries on a "no-cure, no-pay" basis. If university staff develop research outputs of interest to industry, private industries should pay a previously agreed proportion of the development costs in exchange for licence agreements. If the research produces no outputs, the research activity will be funded through the general research budget of the university.

(c) Incubator projects to stimulate and reward talented researchers. These projects would basically serve to "incubate" a promising researcher by supplying financial resources for developing a product and providing technical assistance on the marketing and economic aspects of the product obtained through research. The necessary funds, technical assistance and evaluation of the projects would be financed by Government, or alternatively through the revolving fund. Incubator projects would allow researchers to continue their work at university, guaranteeing the researchers' academic careers while simultaneously allowing them to establish their own company.

In all three cases, successful outcomes or products will become the intellectual property of the private company involved (in cases [a] and [b]) or the individual researcher (in case [c]). If an established private company is involved (cases [a] and [b]), part of the development costs are to be paid by the companies in exchange for a licence agreement. In all three cases, companies must pay royalties to the revolving fund the successful sale of products, as indicated above.

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