Biotechnology in Pakistan

Status and Prospects

Dr. Kauser Abdulla Malik

PAKISTAN ACADEMY OF SCIENCES

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FOREWORD

The tremendous advances in biotechnology have had a profound impact in agriculture, medicine and environment. About 20% of all world pharmaceuticals are produced today by using biotechnological processes and it has been estimated that about 50% of all pharmaceuticals will be produced in this manner by the year 2020. For example, the corticosteroids used in medicine are produced by using certain microbes to introduce a hydroxyl group at the 11-position of the steroidal skeleton. Similarly, insulin used in diabetes is produced by biotechnological processes. Many antibiotics are also manufactured using genetically modified organisms. "Personalized medicine" is being developed, so that medicines will be tailored to match the genetic profiles of various sections of populations.

Biotechnological methods have been used to develop new varieties of crops with better nutritional qualities, that are resistant to environmental stresses and attacks by pests. Genetically modified crops, such as Bt cotton, have been developed that can produce their own pesticides internally. Vitamin A deficiency causes night blindness in about half a million children each year because of lack of vitamin A in their diets. Microbes can also be used to remove toxic wastes and oil from oil spills in the oceans. Golden rice has been produced that contains a precursor of vitamin A to tackle the issue of night blindness in children. These are only some of the thousands of applications of biotechnology in agriculture, health, environment and material sciences.

The present volume authored by Prof. Kauser Malik on behalf of the Pakistan Academy of Sciences highlights the status of biotechnology in Pakistan and points the way to what must be done in the future. It represents the second booklet to be prepared by the Academy on an important area of science, the previous one being on energy. I hope that these documents will be employed by our Planning Division to prepare robust development programmes in the fields identified, so that we can embark on the road to developing a knowledge economy.

I would like to thank Prof. Kauser Malik for devoting time and effort as well as Dr. Abdul Rashid for carefully editing the material.

Atta-ur-Rahman, *FRS, NI, HI, SI, TI*
President
Pakistan Academy of Sciences

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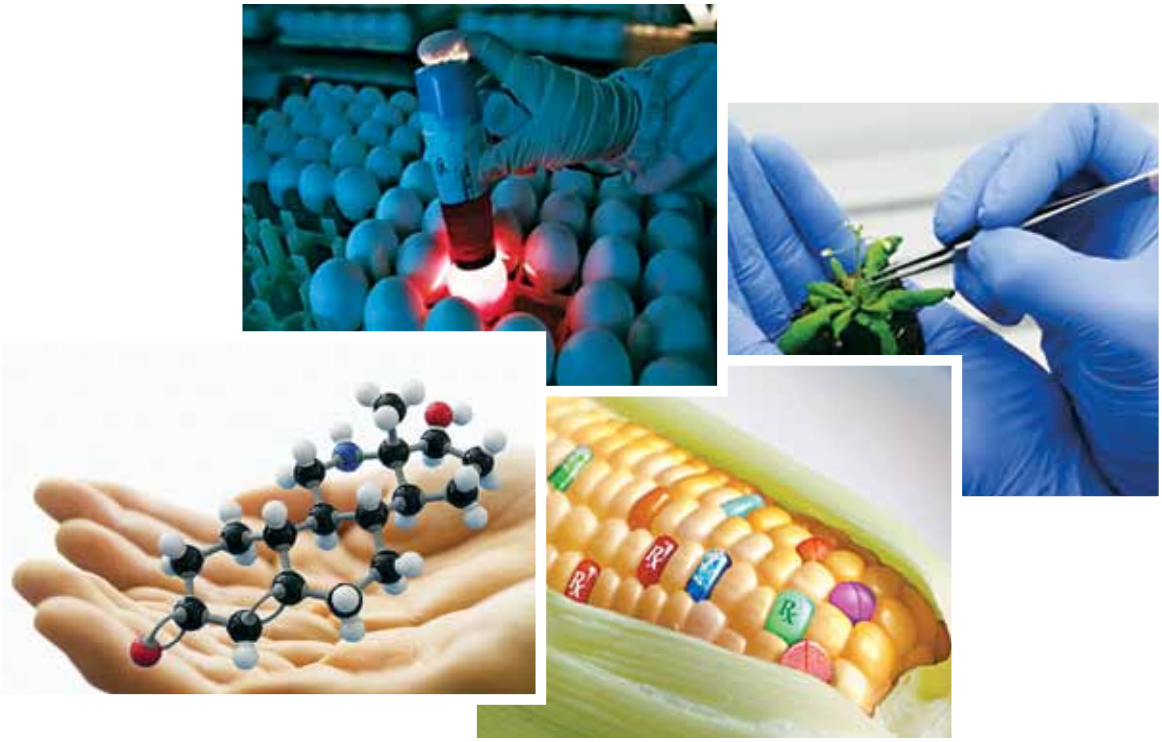

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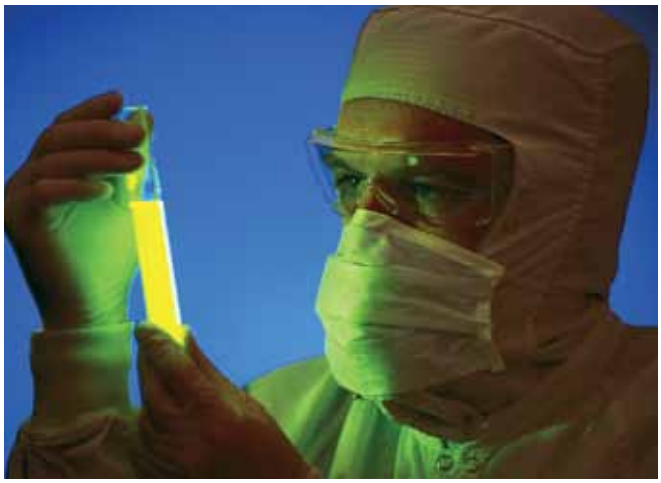

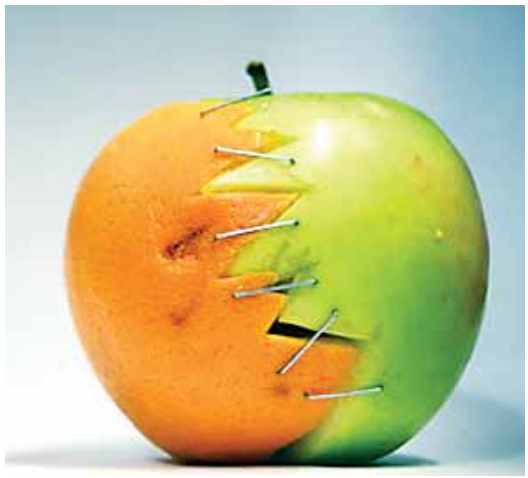

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ABBREVIATIONS AND ACRONYMS

AARI	Ayub Agricultural Research Institute
ABI	Agricultural Biotechnology Institute
ASAB	Atta-ur-Rehman School of Applied Biosciences
BGA	Blue-green algae
BPD	Biological Production Division
CABB	Centre of Agricultural Biochemistry and Biotechnology
CCRI	Central Cotton Research Institute
CFC	Common Fund for Commodities
CLCV	Cotton Leaf Curl Virus
CPB	Cartegena Protocol on Biosafety
CSF	Competitive Support Fund
DIC	Differential Interference Contrast
EDS	Egg Drop Syndrome
EMRO	East Mediterranean Regional Organization
EPI	Expanded Program on Immunization
FAO	Food and Agriculture Organization
FMD	Foot and Mouth Disease
FPLC	Fast protein liquid chromatography
G-CSF	Granulocyte Colony Stimulating Factor
GMOs	Genetically modified organisms
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HEC	Higher Education Commission
HEJ	Husein Ebrahim Jamal
HPLC	High performance liquid chromatography
HRD	Human resource development
IAEA	International Atomic Energy Agency
IBB	Institute of Biochemistry and Biotechnology
IBC	Institutional Biosafety Committee
IBGE	Institute for Biotechnology and Genetic Engineering
ICAC	International Cotton Advisory Committee
ICCBS	International Center for Chemical and Biological Sciences
ICGEB	International Center for Genetic Engineering and Biotechnology
IPO	Intellectual Property Organization
IPR	Intellectual Property Rights
JICA	Japanese International Cooperation Agency
KIBGE	Dr. A.Q. Khan Institute of Biotechnology and Genetic Engineering

LMOs	Living modified organisms
LRBT	Layton Rahmatulla Benevolent Trust
MAS	Marker assisted selection
MFGE	Motor fuel grade ethanol
MNCs	Multi-national companies
MoST	Ministry of Science & Technology
NARC	National Agricultural Research Center
NBC	National Biosafety Committee
NCB	National Commission on Biotechnology
NCBI	National Center for Biotechnology Information
NCEMB	National Centre of Excellence in Molecular Biology
NCVT	National Coordinated Varietal Trials
NGO	Non-governmental organization
NIBGE	National Institute for Biotechnology and Genetic Engineering
NIGAB	National Institute for Genomics and Advanced Biotechnology
NIH	National Institute of Health
NPV	Nuclear polyhedrosis virus
PAEC	Pakistan Atomic Energy Commission
PARC	Pakistan Agricultural Research Council
PCMDR	Dr. Panjwani Center for Molecular Medicine and Drug Research
PCSIR	Pakistan Council for Scientific and Industrial Research
PCST	Pakistan Council for Science and Technology
PCR	Polymerase Chain Reaction
PDP	Plant-derived pharmaceutical protein
PIEAS	Pakistan Institute of Engineering and Applied Sciences
PSF	Pakistan Science Foundation
PVC	Poultry Vaccine Centre
QTLs	Quantitative Trait Loci
SBS	School of Biological Sciences
SME	Small and Medium Enterprise
SWOT	Strengths, Weaknesses, Opportunities and Threats
TAC	Technical Advisory Committee
TIRF	Total Internal Reflection Fluorescence
TRIPs	Trade Related Intellectual Property Rights
UAF	University of Agriculture, Faisalabad
VRI	Veterinary Research Institute
WHO	World Health Organization

EXECUTIVE SUMMARY

Biotechnology is generally defined as application of living systems based technologies to develop commercial processes and products. Over the last few decades, several fundamental discoveries in life sciences have given rise to Modern Biotechnology which is now one of the fastest growing areas of science; hence this century has rightly been termed as 'Century of Biology', hoping that such advances in life sciences will yield changes more momentous than those of electricity and computers. In view of these developments, Biotechnology was included among the six priority areas of Science & Technology by the National Commission of Science & Technology.

An Action Plan to develop Biotechnology was part of the Mid Term Development Framework (2005-2010) of the Planning Commission of Pakistan. Accordingly, more than Rs 2.0 Billion (ca. US$ 40 Million) have been spent over the last few years through HEC, Ministry of Science & Technology and Ministry of Agriculture for developing the infrastructure and capacity building for undertaking R&D in Biotechnology especially related to agriculture and health in various universities and R&D institutes. Several hundred young researchers were sent abroad to various universities of North America and Europe to do PhD or post doctoral research. At present nearly 460 scientists, including more than 200 PhDs, are working in various universities and R&D institutes. Presently, there are nearly 30 centers of Biotechnology spread all over the country.

Nearly all the Biotech Centers are in the public sector and are at different stages of development. The ones in the universities have been essentially involved in the development of human resource. However, only a few institutions have reached a stage where they have some deliverable products. Most of the achievements are in the area of **agricultural biotechnology**. Tissue culture technology has been widely developed and also commercialized. Significant among these are the development of virus free potato and banana. Both of these crops suffer from acute viral infections resulting in colossal economic losses. Use of these technologies has greatly helped in saving these crops. In addition, tissue culture has been used for production of exotic orchids and cut flowers. This technology also holds a great potential in forestry and micropropagation of medicinal and vegetative crops.

Several institutes and university Biotechnology departments have developed biofertilisers based on different non pathogenic microbes responsible for enhancing plant growth by their ability to convert atmospheric nitrogen to ammonium which can be used by plants, by solubilizing insoluble phosphorus and by providing growth hormones to the plant roots. Many such strains have been made in to different formulations and marketed by several agri-business companies under their own trade names.

Major technology development has however occurred in case of genetic engineering in different crops for different traits. This was possible after the establishment of Biosafety Rules and Guidelines incorporated in the Environment Protection Act in 2005. The Biosafety Rules and Guidelines are comprised of three tiers, namely Institutional Biosafety Committee (IBC), Technical Advisory Committee (TAC), and National Biosafety Committee (NBC). All institutions, both public and private involved in R&D in Biotechnology, are required to establish IBC which is responsible for giving clearance for initiating research according to the Biosafety Guidelines. Uptill now nearly 40 IBCs belonging to both private and public sector have been registered with the NBC.

Cotton is one of the most important cash crop for Pakistan. This crop consumes nearly 80% of the pesticide. Therefore pest resistance is one of the most desirable traits in cotton. Following all the requirements of Biosafety Guidelines, in 2010 some varieties of *Bt*-Cotton were released for commercial cultivation. Some of these varieties were developed by private sector seed companies. It is estimated that nearly 2.4 million ha is under *Bt*-Cotton varieties. However major thrust of R&D is for developing Cotton Leaf Curl Virus (CLCV) resistant cotton varieties. Two institutions, namely National Institute for Biotechnology and Genetic Engineering (NIBGE) and National Centre of Excellence in Molecular Biology (NCEMB), are actively involved in this project. Work on several approaches involving recombinant Deoxyribose Nucleic Acid (DNA) technology is being carried out resulting in some success. Hopefully, in near future CLCV resistant cultivars will be available for commercial cultivation.

In addition to cotton, work involving genetic engineering is being done on other crops, namely wheat, sugarcane, potato, tomato, chickpea and some vegetables. In consideration of the problems of our agriculture, major emphasis is on developing salinity and drought tolerant varieties in addition to tolerance to biotic stresses. Emphasis is also being given to improve the nutritional quality of our staple crops, especially wheat, in view of the fact that the National Nutrition Survey of 2011 revealed acute deficiencies of iron and zinc in our diet.

In the area of **health** most of the efforts have gone into determining the prevalence of some human diseases, and scoring the type and nature of mutations (both somatic and genetic) in genes directly or indirectly involved in specific human diseases and genetic disorders, such as partial or complete hearing or vision loss, skin diseases, polydactyly, mental retardation, different types of cancers, etc. In addition, work has been carried out essentially at NIBGE and NCEMB on developing PCR based diagnostic protocols for various diseases such as Tuberculosis (TB), Typhoid, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and some of the cancers. In case of Biologicals, NCEMB and SBS at University of the Punjab have been able to produce Interferon, Human Growth Hormones and Insulin at laboratory scale. Stem Cell Technology has also been developed in laboratories of Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), NCEMB and Atta-ur-Rehman School of Applied Biosciences (ASAB). However, this technology should be developed as a major research area for specific applications in the field of human health.

With dwindling resources of fossil fuel, the demand for production of bioethanol and methane gas from plant biomass is gaining more and more importance. A mega project funded by the Planning Commission was initiated on “Production of Bioenergy from Plant Biomass”. It is being executed at the School of Biological Sciences (SBS) of University of the Punjab, the Industrial Biotechnology Institute of Government College University, Lahore, and Pakistan Council for Scientific and Industrial Research (PCSIR), Lahore. Several enzymes involved in the bioconversion of lingo-cellulosic biomass have been characterized and cloned for over-expression and large-scale production of the selected enzyme activities so that these can be used for the saccharification of celluloses and hemicelluloses of plant biomass. Once this is achieved, the sugars thus produced can be used in a fermentation process to produce alcohol. Similar strategies can be used for anaerobic conversion of biomass to methane gas which can be converted to electricity. These are the strategies which hold a lot of promise and must be used for converting the waste to energy.

Biotechnology is also a main contributor to Small and Medium Enterprise (SME) development by encouraging establishment of Start Up companies. Such an activity can be facilitated by active

participation of the Government by providing financial support for such companies because of the absence of Venture Capitalists. In order to achieve this, it is suggested that a Venture Capital Fund be established with a contribution by the Government represented by Higher Education Commission (HEC) and Ministry of Science & Technology (MoST) and private sector represented by the Federal Chamber of Commerce and Industry. Such a fund would go a long way in commercializing of Biotechnology.

This report has endeavored to document the status of Biotechnology in the country. There was a liberal support for developing Biotechnology during the previous decade. Necessary infrastructure has been provided and ambitious HRD programs were launched. Presently, a critical expertise in all the related areas of Biotechnology is available. However, inspite of all these efforts, present investment on Biotechnology remains sub-critical. This has been further compounded by the current economic crisis resulting in slashing or delaying of a number of Biotechnology related developmental projects. Therefore, at this juncture any additional investment into this sector will be most productive. However, it is necessary to review the status of Biotechnology R&D in the country and its potential for commercialization. It is also worth mentioning that the present Federal Government of the Pakistan Muslim League (N) has mentioned Biotechnology and Nanotechnology as the priority areas of Science & Technology in their Manifesto with a plan to establish Foundations for both of these disciplines.

1. INTRODUCTION

Biotechnology has been defined in many forms but, in essence, implies the use of microbial, animal or plant cells or enzymes to synthesize, breakdown or transform material for production of goods and services to the mankind. It is a multidisciplinary field of activity, which heavily relies on integration of Biology, Biochemistry, Microbiology, Molecular Biology, Chemical Engineering and Process Engineering, together with other disciplines, in a way that optimizes the exploitation of their potential. Biotechnology in itself is not a product or a range of products like Microelectronics; rather it is regarded as a range of enabling technologies which find significant application in several sectors. By virtue of being multidisciplinary in nature, the scope and opportunities of Biotechnology are wide and varied in the areas of agriculture, industry, food and beverages, health, environment, and energy.

Over the last few decades, several fundamental discoveries in life sciences have given rise to modern Biotechnology which is now one of the fastest growing areas of science; and this century has rightly been termed as 'Century of Biology', hoping that such advances in life sciences will yield changes more momentous than those of electricity and computers.

With the opening up of the country's economy and liberalization, Pakistan Government has taken many steps to reorient it, and to move towards a knowledge-based economy. Biotechnology has thus been included in the six priority areas of Science & Technology Policy of the country. In order to further develop this technology, a National Commission on Biotechnology was established with the objective of advising the Ministry of Science & Technology (MoST) and to help monitor new developments in the field of Biotechnology at national and international levels. A National Policy and Action Plan has been developed which is now part of the Mid Term Development Framework (2005-2010) of the Planning Commission of Pakistan. In accordance with this Action Plan, more than Rs. 2.0 Billion (ca. USD 40 Million) have been spent over the past few years for developing the infrastructure and capacity building for undertaking R&D in Biotechnology (Appendix 1). At present there are nearly 500 scientists conducting Biotechnology research at 30 universities and R&D organizations of the country (Figure 1; Table 1).

Figure 1. Location of the Biotechnology Institutes in Pakistan.

Table 1. Institutes/Departments of Biotechnology and Genetic Engineering in Pakistan.

S. #	Institute/Department	Organization/University	City	Phone #	Faculty Members
1.	National Institute for Biotechnology and Genetic Engineering (NIBGE)	Pakistan Atomic Energy Commission (PAEC)	Faisalabad	041-2651475	57
2.	Nuclear Institute for Agriculture and Biology (NIAB)	Pakistan Atomic Energy Commission (PAEC)	Faisalabad	041-2654210	76
3.	Centre of Agricultural Biochemistry and Biotechnology (CABB)	University of Agriculture	Faisalabad	041-9201087	7
4.	Punjab Agricultural Biotechnology Institute	Ayub Agricultural Research Institute (AARI)	Faisalabad	041-2550715	42
5.	National Centre for Excellence in Molecular Biology (NCEMB)	University of the Punjab	Lahore	042-5293141-47	26
6.	Institute of Biochemistry and Biotechnology (IBB)	University of the Punjab	Lahore	042-9230355	6
7.	School of Biological Sciences (SBS)	University of the Punjab	Lahore	042-9230970	10
8.	Department of Microbiology and Molecular Genetics	University of the Punjab	Lahore	042-9231248	18
9.	Industrial Biotechnology Institute	Government College University (GCU)	Lahore	042-9211634	8
10.	Biotechnology and Food Research Center	Pakistan Council for Scientific and Industrial Research (PCSIR) Laboratories	Lahore	042-9230704	11
11.	Cytogenetics Section	Central Cotton Research Institute (CCRI)	Multan	061-9200340	1
12.	Agricultural Biotechnology Institute (ABI)	National Agricultural Research Center (NARC)	Islamabad	051-9255012	12
13.	National Institute of Genomics and Advanced Biotechnology (NIGAB)	National Agricultural Research Center (NARC)	Islamabad	051-9255680	8
14.	Biomedical and Genetic Engineering Division	Dr. A.Q. Khan Research Laboratories	Islamabad	051-9261138	3
15.	Department of Biological Sciences	Quaid-i-Azam University	Islamabad	051-90643703	24
16.	Institute of Bioinformatics	Quaid-i-Azam University	Islamabad	051-90643003	Under process
17.	Department of Biotechnology	Quaid-i-Azam University	Islamabad	051-90643073	5
18.	Atta-ur-Rehman School of Biosciences (ASAB)	National University of Sciences and Technology (NUST)	Islamabad	051-111-11-6878	3
19.	Department of Biotechnology	University of Karachi	Karachi	021-9261300-06, Ext: 2474	18
20.	Center for Molecular Genetics	University of Karachi	Karachi	021-4966045	3
21.	Department of Microbiology	University of Karachi	Karachi	021-9261300-06, Ext: 2248	12
22.	Dr. Punjwani Center for Molecular Medicine and Drug Research (PCMDR)	University of Karachi	Karachi	021-4824924-25	13

(Contd.)

Table 1 (Contd.)

S. #	Institute/Department	Organization/University	City	Phone #	Faculty Members
23.	Husein Ebrahim Jamal (HEJ) Research Institute of Chemistry	University of Karachi	Karachi	021-4824901-2	37
25.	Dr. A.Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE)	University of Karachi	Karachi	021-4823889	14
25.	National Center for Proteomics	University of Karachi	Karachi	021-9261300-06 Ext:2413	5
26.	Institute of Biotechnology and Genetic Engineering (IBGE)	University of Sindh	Jamshoro	022-9239024 022-2772359 0303-6142398	10
27.	Institute of Biotechnology and Genetic Engineering (IBGE)	KPK Agricultural University	Peshawar	091-9216572-79	8
28.	Center for Animal Biotechnology	Veterinary Research Institute, KPK	Peshawar	091-9210218-19	4
29.	Center of Biotechnology	University of Peshawar	Peshawar	091-9216701	2
30.	Institute of Biochemistry	University of Balochistan	Quetta	081-9211261	8
31.	University Institute of Biochemistry and Biotechnology	PMAS-Arid Agriculture University	Rawalpindi	051-9219809	7
	Total Number				**458**

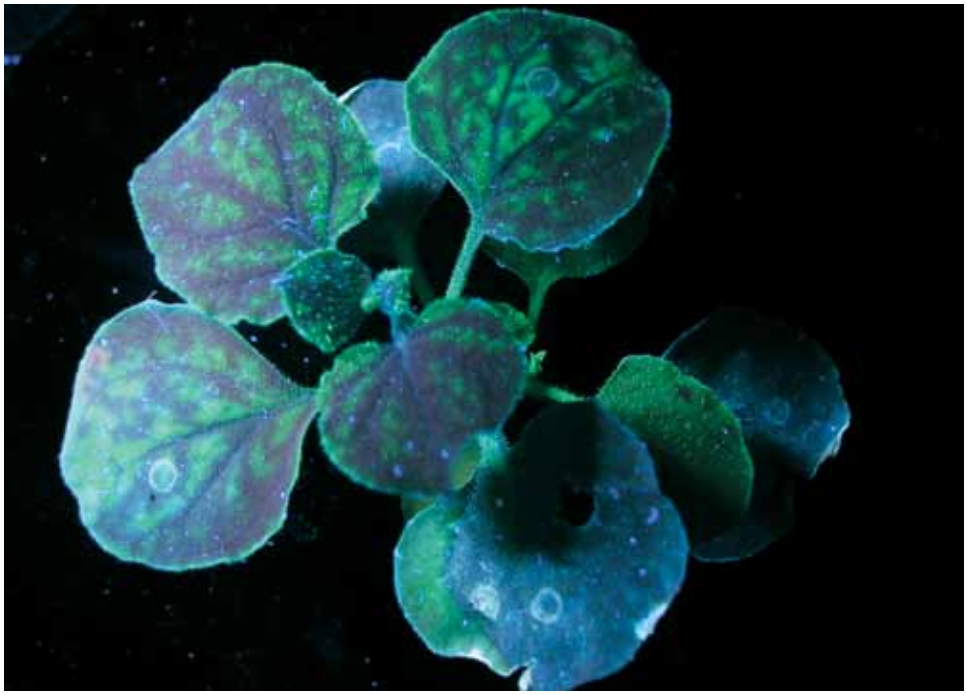

Figure 2. Green Fluorescent Protein (GFP) being expressed in Tobacco *(Nicotiana benthemiana).*

2. PROFILE OF KEY BIOTECH INSTITUTES

The Biotech Centers in the public sector are at different stages of development. The ones in the Universities have been essentially involved in the development of human resource. However, there are only a few which have some deliverable products and services. Profiles of these Institutes are given below:

2.1. NATIONAL INSTITUTE FOR BIOTECHNOLOGY AND GENETIC ENGINEERING (NIBGE), FAISALABAD

Figure 3. National Institute for Biotechnology and Genetic Engineering, Faisalabad.

The National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad is a federal research institute, which was established in 1992. Within a short span of time, this Center has earned a place among the scientific institutions of excellence in the country and is well recognized internationally. NIBGE has been awarded the status of an Affiliate Center of the International Center for Genetic Engineering and Biotechnology (ICGEB).

There are five research Divisions in NIBGE, namely Agricultural Biotechnology, Health Biotechnology, Industrial Biotechnology, Environmental Biotechnology and Technical Services. An overview of their research activities is available at www.nibge.org.

Agricultural Biotechnology Division of NIBGE has so far submitted around 40 cases of laboratory manipulation, field trials and commercial release of genetically modified (GM) crops to the National Biosafety Committee (NBC). Most of these cases deal with introduction of single gene traits for control of insect pests, viruses and resistance against abiotic stresses, such as salt, drought and heat tolerance. These are termed as **"First Generation GM Crops**" One non-GM and two GM cotton varieties have been approved already. In the "**Second Generation GM Crops**", technologies that depend upon

expression of 2-3 genes are being developed. However, NIBGE plans to move to the "**Third Generation GM Crops**" where more than three genes are expressed for "stacked characters" and the expression of the transgene may be limited to the tissues where it is required; for example, roots, phloem, seeds, etc.

Figure 4. Real Time PCR for Gene Expression Analysis.

Figure 5. Automated DNA Sequencer for Nucleotide Sequence Determination.

The status of some of the GM crops developed by NIBGE, Faisalabad is given in Tables 2(a), 2(b), 2(c), 2(d).

Table 2(a). The Desirable Traits and Genes used for Cotton Improvement at NIBGE, Faisalabad.

Trait	Gene/Cotton Variety	Status
Virus tolerance/ Insect resistance	1. IR-NIBGE-1524 (Bt MON531) 2. IR-NIBGE-3701(Bt MON531) 3. IR-NIBGE-901 (Bt MON531) 4. IR-NIBGE-3 (Bt MON531) —2012 5. IR-NIBGE-4 ((Bt MON531) 6. IR-NIBGE-5 (Bt MON531)	Approved in 2006 Approved in 2010 for Punjab, 2011 for Sindh Approved in 2010 for Punjab, 2011 in Sindh (highly popular in Punjab and Sindh) Approved in 2011 (highly popular in Sindh) Approved in 2012 in Punjab Under field trial/approval process
Bollworm resistance	1. Cry1Ac 2. Hvt 3. Cry2Ab 4. Cry1Ac + Cry2Ab 5. Cry1Ac+Cry2Ab+EPSPS 6. Vip3A	1. Transgenic Cry1Ac cotton developed – shared with NIAB 2. Hvt cotton developed – biosafety in progress 3. Transformation stopped due to effect on embryogenesis 4. Second generation Bt cotton having two genes, transformation in progress 5. Two Bt genes generation Bt cotton having two genes, transformation in progress
Virus (CLCD)	1. NIBGE-2 (marker-assisted selection) 2. NIBGE-115-2012 3. Rep antisense 4. Truncated Rep (2 constructs) 5. RNAi (βC1) 6. RNAi (V2) 7. GroEL+Zinc finger+G5 8. Artificial microRNA 9. Amplicon rep RNAi 10. Amplicon beta C1 RNAi	1. Approved in 2006 and used as a parent for IR-NIBGE-3701 2. Approved as variety in 2012 3. Transgenic plants showed partial resistance in the field 4. Failed to offer resistance under field conditions 5. Partial resistance under field conditions 6. Partial resistance under field conditions 7. Two versions of triple gene cassette were transformed into Coker 312. 8. Twenty events were produced which are on embryogenesis medium 9. One micro-RNA construct and amplicon constructs 10. targeting cotton leaf curl virus (CLCV) were transformed into Coker 312 and various events are on embryogenesis medium
Fiber	1. β-1,3-endoglucanase RNAi 2. Expansin 3 (ExpA3) 3. Aquaporin 2 (PIP2) 4. Sucrose synthase	1. Transgenic cotton developed. Some of the transgenic lines showed improved fiber length. Ready for introgression. 2. Transgenic cotton, under development 3. Transgenic cotton, under development 4. Transgenic cotton, under development
Abiotic stresses: i) Drought ii) Salt tolerance	1. AVP1 2. AtNHX1	1. Transgenic (*AVP-1*) cotton was developed. The transgenic germplasm was provided to NIAB, NIA and NIBGE for introgression into local elite cultivars for the improvement of drought tolerance, improved germination and phosphorus uptake. 2. *AtNHX1* gene was transformed into Coker 312 for developing salt tolerant cotton, and transgenic cotton plants were developed. These transgenic lines are being characterized for salt tolerance

Table 2(b). The Desirable Traits and Genes used for Wheat Improvement at NIBGE, Faisalabad.

Trait	Gene/Wheat Variety*	Status
Phosphorus / Nitrogen use efficiency	1. AVP1 2. AlaT	1. Transgenic (AVP1) wheat showed better growth on P deficient soils. 2. Synthetic *AlaT*gene developed and cloned in plant transformation vectors and transformation of wheat (local varieties) in progress
Heat tolerance	1. AVP1 / AVP1-D 2. HVA1	Trials under progress following late sowing under field conditions.
Drought/Salt tolerance/Heat	1. AVP1-D 2. HVA1 3. DREB1A 4. AtNHX1 5. AtNCED3 6. WXP1 7. HSR1	1-6. Six different genes were transformed into local elite cultivars of wheat. Limited field trials of some of the above mentioned transgenic lines are in progress at seven locations across Pakistan. 7. HSR1 plants showed better performance under drought and heat stress
Rust resistance	Rust resistant varieties (*Seher,* Punjab-11 and one line from NIA) used in transformation	Some basic work on identification of rust resistance gene was done, however, no gene is available yet for rust resistance.
Herbicide tolerance	CP4 *epsps*	Transformation of local elite cultivars has been started recently.

*All transformations were done in two wheat cultivars, i.e., *Seher*-2006 and Punjab-2011.

Table 2(c). The Transgenic Sugarcane Lines Developed at NIBGE.

Trait	Gene/Sugarcane Variety	Status
Insect resistance	Cry1Ac + cry2Ab	Synthetic *cry1Ac* + *cry2Ab* transformed into sugarcane for resistance against sugarcane top borer. Yet to be tested under field conditions
Drought / Salt / Frost tolerance	1. AVP1	1. Transgenic AVP1 plants of four cultivars developed. 3-5 events of each cultivar showed drought tolerance (60% irrigation). Field experiments for drought tolerance are being conducted at different locations
	2. AtNHX1	2. Transgenic AtNHX1 plants of four cultivars developed. 3-5 events of each cultivar showed salt tolerance upto 150 mM. Field experiments for salt tolerance are being conducted at different locations
	3. DREB1A	3. Transgenic DREB1A plants of four cultivars developed. 3-5 events of each cultivar showed tolerance to low temperature i.e. exposure to cold (at 4 °C) for 15 days

Table 2(d). The Transgenic Potato Lines Developed at NIBGE, Faisalabad

Trait	Gene/Potato Variety	Status
Late blight	BLB gene isolated from a wild potato	Transformed into potato. Transgenic plants at selection phase. Initial screening shows that plants are resistant to late blight
Virus resistance	1. CP RNAi (PVX, PVY, PLRV) 2. NTP (PVX), HcPro(PVY), CP (PLRV) / RNAi 3. CP(PVX,PVYPVS)/RNAi 4. CP PLRV+ ToLCNDV/RNAi 5. RNAi silencing of vacuolar invertase	1. Transgenic potato failed due to presence of mixed infection of PVS, PVA and PVM in the field 2. Transgenic potato failed due to presence of mixed infection of PVS, PVA and PVM in the field 3. Plants grown in tunnel conditions 4. Plants grown in tunnel conditions 5. Plants grown in tunnel for tubers
Frost tolerance	1. AVP1 2. DREB1A 3. hsr1	Transformation of synthetic *AVP1, DREB1A* and*hsr1* genes for abiotic stress tolerance are in progress.

Cotton crop is backbone of the Pakistan's economy. Therefore, in the Agricultural Biotechnology Division, major emphasis has been on cotton Biotechnology especially on the cotton leaf curl virus (CLCV) disease. The relationship of Gemini viruses with cotton leaf curl disease has been understood at molecular level and *Burewala* strains-linked disease has been tackled by developing the ways to differentiate between different viral genomes. A tripartite project with the John Innes Centre, Norwich, UK and the University of Arizona, USA, through the International Cotton Advisory Committee, Washington DC, USA, helped in understanding and developing new strategies to combat this disease. Based on the information provided by the molecular characterization of the causative virus, a Group in the Plant Biotechnology Division is engaged in the development of virus resistant cotton through genetic engineering. The transgenic cotton is being evaluated in containment facilities of NBGE. This facility is first of its kind in the country and will pave the way for commercial release of genetically engineered plants. In addition, a cotton genome project has been initiated with the objective of identifying various genes of agronomic importance.

In addition, work on other crops, such as potato, sugarcane, tomatoes and chillies, is also going on.

Figure 6. An array of Green House and Containment Facilities at NIBGE, Faisalabad.

In the Plant Microbiology laboratories of NIBGE, work is being carried out on various microbial inoculants for enhancing plant growth. A Biofertilizer comprising of a consortium of different beneficial known and characterized bacteria has been developed and commercialized under the trade name *BioPower* for various crops. Its use not only reduces the input of chemical fertilizers but also enhances the yields to significant levels.

In the area of health, NIBGE has been a pioneer in developing diagnostic tools for various diseases based on Polymerase Chain Reaction (PCR). In-house PCR/ DNA-based diagnostic tests for Tuberculosis and Hepatitis C were developed by the NIBGE scientists in 1993 and 1995, respectively. And the PCR tests for β-thalassemia and Typhoid PCR were developed in 1996 and 1997, respectively. Later on two PCR tests were developed for Hepatitis-B and Leukemia (bcr-abl transcript). Diagnostic services of these tests were offered to the public and the research findings pertaining to these tests were presented in international and national conferences and international publications.

Biomining of low grade Pakistani ores is another R&D area ready for commercial application. In this regard pilot scale model projects have been developed for biotechnological recovery of uranium, copper, gold, etc. It has been estimated that the value of recoverable quantities of different metals is about 6,000 Million US Dollars. For copper alone recovery of the metal from Sandak (Balochistan province), over the burden, is worth about 80 Million US Dollars. Adoption of these technologies on national level will yield a huge economic impact besides creating job opportunities and fortifying Government's efforts for poverty eradication. Similarly, upgradation of fossil fuels for energy generation and general use is a sought after area of R&D. Coal biodesulfurization is a highly attractive alternative for economic gains besides being an environmental friendly technology. The NIBGE scientists have developed a coal desulfurization process at pilot scale which has recently been handed over to the cement industry for commercial exploitation. It has been estimated that this process is much cheaper and less labor intensive than the chemical and physical processes being used at present. Desulfurization of coal by this process saves about 500 rupees per ton and also enhances its calorific value to a great extent.

Similarly, efforts have been put to design processes to combat environmental pollution/threats for various industries as well as for urban environment in general. In Environmental Biotechnology Division, processes based on microbial detoxification of industrial effluents have been developed and an active collaboration with the private sector has been established. Even Genetically Modified Bacteria have been developed to handle the environmental problems, such as treating of sewerage and oil spills, like from the Greek ship MV Tasman Spirit at the Karachi port. The GM bacteria convert crude oil and gasoline into non-toxic substances such as carbon dioxide, water and oxygen and help create a cleaner, healthier environment.

The work on Biofuels (i.e., Bio-diesel and Bio-ethanol) has also reached a stage where upscaling of the process for microbial conversion of plant biomass (straw, bagasse, etc.) into methane and alcohol has become feasible.

The NIBGE laboratories have state-of-the-art equipment including capillary electrophoresis based automated DNA sequencers (Perkin Elmer), fomenters, a DNA synthesizer, FPLC, HPLC, Transmission Electron Microscope, Scanning Electron Microscope, Atomic Force Microscope, Confocal Microscope, GC/MS, LCMS high speed centrifuges, gel electrophoresis system, etc. The NIBGE's library has been declared as the National Library for Biological Sciences and subscribes to about 30 scientific journals as well as Current Contents on diskette. NIBGE is formally affiliated with the Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad for conferring of MPhil and PhD degrees in Biotechnology.

Figure 7. Electron Microscope for Ultrastructure Determination.

Figure 8. Atomic Absorption Spectrometer for Characterization of Volatile Materials.

Information about the commercial services and products developed by NIBGE is given in Table 3. The patents approved or filed by the NIBGE scientists have been listed in Table 4 and the information about the patents approved or filed in other countries in which the NIBGE scientists are co-authors is presented in Table 5.

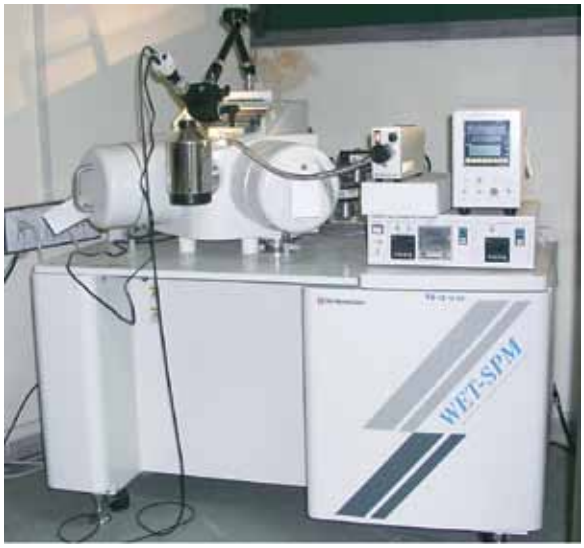

Figure 9. Atomic Force Microscope for Characterization of Nanoparticles.

Figure 10. 10-Liter Fermentor for Biofuel Production.

Table 3. The Commercial Services and Products Developed by NIBGE, Faisalabad.

S. #	Product/Service	Status	Capacity	Client History	Potential Customers
1	NIBGE-2 Cotton	Approved	--	Yet to be established	As the variety is tolerant to virus disease and drought, its Bt version will catch area
2	NIBGE-115 Cotton	Second year of National Coordinated Varietal Trials (NCVT)	--	Yet to be established	The variety is extremely tolerant to *Burewala* virus disease, and will spread among the farmer community after its approval
3	Coker Bt Cotton	Case pending with Technical Advisory Committee (TAC) of National Biosafety Committee (NBC)	--	Provided to NIAB, Faisalabad	The trait is highly desirable and will provide legal commercialization of Bt cotton
4	Bt Tobacco	Case approved by Institutional Biosafety Committee (IBC)	--	Yet to be established/ Government of Pakistan, Punjab and Sindh	Major commercial crop in KPK; good impact on environment/cost of production by tobacco farmers
5	Gemini virus resistant Tomato	Case approved by IBC	--	Yet to be established/ Government of Pakistan, Punjab and Sindh	Virus problem is the major limiting factor and virus resistant tomato will be useful for the whole country
6	Virus free Seed Potato	Virus-free seed potato produced	50,000 mini tubers/ year	Zaib Seed, Jhang and Yousaf Ali and Sons, Gilgit	Seed potato producers
7	DNA testing service for Basmati Rice	Basmati rice exporters	All tests requested	Rice Exporters	Rice exporters/Rice importers
8	GMO testing service	Exporters/importers of agricultural products	All tests requested	Exporters/importers of agricultural products	Exporters/importers of agricultural products/ Government agencies and seed companies
9	Coker HVT Cotton	Approved by IBC	--	Yet to be established	Cotton growers/cotton seed companies
10	Virus resistant Coker Cotton	Approved by IBC	--	Yet to be established	Cotton growers/cotton seed companies
11	Ethanol-Culture (Yeast)	Established	-	Shakargunj Sugar Mills, Jhang; Habib Sugar Mills, Nwabshah	Research Institutes/Industry
12	Yeast Extract	Established	-	R & D	Research Institutes
13	Citric Acid	Established	-	R & D	SMEs
14	Methane-Culture	Established	-	Habib Sugar Mills, Nawabshah	Alternate Energy Source-SME.
15	Xylanase	Established	-	National feed industry	Animal Feed industry
16	Cellulas-less Xylanase	Established	-	Packages Ltd., Lahore	Paper & Pulp industry

(Contd.)

Table 3 (Contd.)

S. #	Product/Service	Status	Capacity	Client History	Potential Customers
17	Cellulase	Established	-	Ruminant/ Animal feed industry	Animal Feed Industry/ Paper & Pulp industry
18	α-amylase	Established	-	Denim & Textile Industry	Denim & Textile Industry
19	Hepatitis C	Established	3000 tests	Medical Practitioners	Medical Practitioners
20	Tuberculosis	Established	1000	-	-
21	CML	Established	500	-	-
22	Karyotyping	Established	100	-	-
23	HBV-DNA PCR Kit	Still to be validated by Diagnostic Laboratories	NA	PCR Diagnostic Laboratories	Molecular Diagnostic laboratories; Research Centers/ Universities
24	*BioPower* – a Biofertilizer for Crops	Already Commercialized	40,000 bags	Several distributors appointed	Local customers & farmers
25	Colliodal Gold or Gold Nanoparticles	Laboratory scale (can be upscaled)	Can be upscaled to tens of liters/day	N/A	Researchers and may also be commercialized as a nanomedicine
26	Colloidal Silver or Silver Nanoparticles	Laboratory scale (can be up scaled)	Can be upscaled to tens of liters/day	N/A	Researchers and may also be commercialized as a nanomedicine
27	Fluoride and Cyanide Analysis	Established	As required	Variable	JDW Sugar Mills Ltd., Lahore; Crescent Textile Mills, Faisalabad. Ali Hajvairy Bottles, Faisalabad; WAPDA, etc.
28	Functional Group Analysis in Organic Compounds	Established	As required	Variable	Assistant Collecter Imports, Faisalabad; Dry Port, Faisalabad; etc.
29	Elemental Analysis	Established	As required	Variable	Chemical industries (Olympia) and various Coal Mining Industries
30	Microbiological Analysis	Established	As required	Variable	Several industries

Table 4. The Patents Approved or Filed by the NIBGE Scientists.

S. #	Authors	Title	Patent /Application No.	Status
1	Mukhtar, Z., Khan, S.A., Zafar, Y. and Malik, K.A.	Synthetic Omega Atracotoxin Hv1a Gene of Funnel Web Spider (*Hadronyche versuta*) for Development of Insect Resistant Plants	Patent No.139032 Application No. 567/2004	Approved
2	Khan, M.S; Ali, S. and Khalid, A.M.	Invitro Direct Regeneration for Sugarcane Leaves (Smart GreencaneTM Seedlings)	No.187658 in class 31	Approved
3	Mukhtar, Z., Khan, S.A., Zafar, Y. and Malik, K.A.	Novel Sequences	Application No. 567/2004.	Submitted to IPO-Pakistan
4	Anwar, M.A., Ghauri, M.A., Akhtar, N., Iqbal, A., Munawar, N., Saleem, M. and Khalid, A.M.	Biodesulfurization of High Sulfur Pakistani Coal by Heap Leaching	Application No. 279/2006.	Submitted to Controller Patents Office, Karachi
5	Mukhtar, Z., Khan, S.A., Zafar, Y. and Malik, K.A.	Synthetic Bt Cry1Ac gene for Effective Control of Insect Pests	Patent Application No. 236/2006	Submitted to IPO-Pakistan
6	Asad, S., Mansoor, S., Zafar, Y. and Malik, K.A.	Induction of Geminiviral Resistance in Cotton Plants	Application No. 429/2008	Submitted to IPO-Pakistan
7	Bashir, A. Khatoon, A. Hifza, M. and Malik, K.A.	Calotropis Procera Aquaporins	-	Submitted to a patent attorney in Lahore

Table 5. The Patents Approved Abroad in which the NIBGE Scientists are Co-authors.

S. #	Authors	Title	Patent /Application No.	Status
1	Maliga, P., Kuroda, H. and Khan, M.S.	Inventors of Invention Entitled, "Translation Control Elements for High Level Protein Expression in the Plastids of Higher Plants and Methods of use Thereof"	US Patent, 2006, No. 6987215 B1	Approved
2	Iqbal, M., Evans, P. and Roberts, S. M.	Synthetic Methods and Novel Chemical Compounds	US Patent, 2005, No. US2005107477 A1	Approved
3	Iqbal, M., Evans, P. and Roberts, S.M.	Cyclopentenone Derivatives, Their Preparations and Use in Medicine	European Patent, 2004, No. EP1487772 A2	Approved
4	Iqbal, M., Evans, P. and Roberts, S.M.	Cyclopentenone Derivatives, Their Preparations and Use in Medicine	World Wide Patent, 2003, No. WO 03/080552 A2	Approved
5	Iqbal, M., Evans, P. and Roberts, S.M.	New Synthetic Methods and Novel Chemical Compounds	Australian Patent, 2003, No. AU2003217021 A1.	Approved
6	Iqbal, M., Evans, P. and Roberts, S.M.	New Synthetic Methods and Novel Chemical Compounds	British Patent, 2002, No. GB0207028D D0	Approved
7	Parvez, S. and Kim, H.Y.	The Methods of Overproducing Bile Salt Hydrolase by co-culturing of *Bifidbacterium bifidum*, and the Composition thereof	Registration Date 2007.02.28, Patent No. 0691839	Approved

(Contd.)

Table 5 (Contd.)

S. #	Authors	Title	Patent /Application No.	Status
8	Parvez, S. and Kim, H.Y.	The Methods of Lowering Cholesterol Absorption by Co-culturing of *Bifidobacterium bifidum* and the Composition thereof	Registration Date 2007.02.28, Patent No. 0691863	Approved
9	Parvez, S., Bae, H.S., Shin, M.K., Hong, M.C., Kang, M.K., Chung, H.S., Cho, C.W. and Lee, S.M.	New Gene and Polypeptides of Platelet derived Growth Factor B	Registration Date 2007.07.18, Patent No. 0742313	Approved
10	Parvez, S., Bae, H.S., Shin, M.K., Hong, M.C., Kang, M.K., Chung, H.S., Cho, C.W. and Lee, S.M.	Lactobacillus Acidophilus PM1 removing Heavy Metals	Registration Date 2007.09.17, Patent No. 0760987	Approved
11	Parvez, S. and Kim, H.Y.	Process for Production of Yeast Extract by Autolysis by means of Temperature Control	Registration Date 2006.10.23, Patent No. 0639847	Approved

2.2. DR. PANJWANI CENTER FOR MOLECULAR MEDICINE AND DRUG RESEARCH (PCMDR), INTERNATIONAL CENTER FOR CHEMICAL AND BIOLOGICAL SCIENCES (ICCBS), UNIVERSITY OF KARACHI, KARACHI

Figure 11. Dr. Panjwani Center for Molecular Medicine and Drug Research, ICCBS, University of Karachi, Karachi.

Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMDR), established in 2004, is now among the most prominent Biotechnology research establishments of Pakistan. The main objective of the Center is to focus on training of students at MPhil and PhD levels in the emerging fields of Molecular Medicine and Drug Research. The Center has 15 faculty members and around 50 MPhil/ PhD students working on research projects relevant to the national health needs with an aim of finding improved and novel ways of their diagnosis, treatment and prevention. The Center has acquired state-of-the-art scientific instruments for world-class research program.

Following is a brief outline of the research activities at various laboratories of PCMDR:

2.2.1. Immunology Laboratory

The Immunology Laboratory is currently involved in screening of compounds for their anti-inflammatory immunosuppressive properties by means of an array of immunological assays. These assays are based on the cellular effects of compounds on cytokines, T-cell proliferation and oxidative burst

(Chemiluminescence Assay). In addition, various cytotoxicity and anti-cancer studies are routinely performed on natural/synthetic products using mouse fibroblast cell line NIH 3T3.

2.2.2. Cell Biology Laboratory

The Cell Biology Research Laboratory is equipped with the state-of-the-art High-resolution Light Microscopy facility capable of performing Bright-field Microscopy, Differential Interference Contrast (DIC) Microscopy, Multi-channel Fluorescence Microscopy, Deconvolution Microscopy and Total Internal Reflection Fluorescence (TIRF) Microscopy. In the Laboratory, routine experiments related to histopathology, immunocytochemistry and immuno-histochemistry are performed on various biological specimens including liver, heart, brain, etc.

Figure 12. Flow Cytometer being demonstrated for the Study of Microbial Cells.

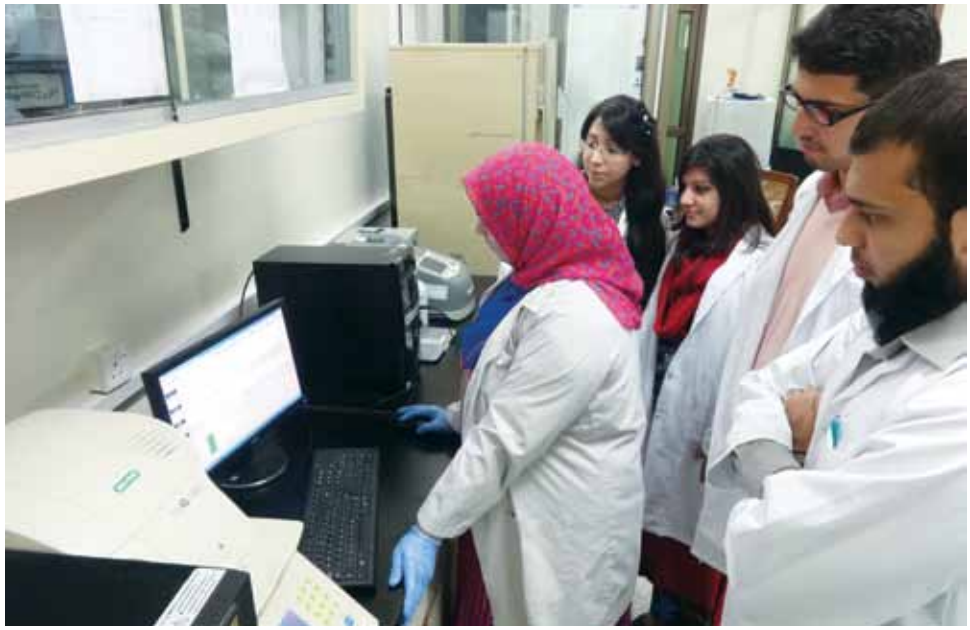

Figure 13. A Biotech Research Group working on RT-PCR for the detection of Hepatitis B & C Viruses.

2.2.3. Stem Cell Laboratory

The Stem Cell Research Laboratory is working towards the objective of establishing a facility that could explore the potential of bone marrow stem cells as regenerative medicine. The experiments being routinely performed here include isolation of bone marrow stem cells, characterization of stem cells and cardiomyocytes by immunocytochemical techniques using fluorescence probes, Western Blotting and Flow Cytometry and extraction of RNA from rodent heart and analyzing them for the expression of cardiac specific proteins by Western Blotting and RT-PCR. In addition, bone marrow cells are being analyzed for the expression of various cytokines after preconditioning them under hypoxic conditions in order to delineate the role of specific cytokines that are involved in homing of these stem cells to the site of injury.

2.2.4. Computational Chemistry Laboratory

The research in this Laboratory involves the application and development of all aspects of medicinal chemistry, organic synthesis, molecular modeling, computational chemistry, computer-aided drug design, virtual screening (docking, scoring, 3D-QSAR; CoMFA, COMSIA), virtual combinatorial library design using pharmacophore approaches, protein structure prediction and molecular dynamics simulations.

2.2.5. Molecular Biology Laboratory

Research at the Molecular Biology Laboratory includes elucidating the role of urinary modulators in urolithiasis, evaluation of herbal preparations for kidney stones, allergies, asthma and inhalant allergens and elucidation of free radical scavengers in animal models.

2.2.6. Molecular Pharmacology Laboratory

Research in this Laboratory includes analyzing the role of lipoproteins and other inhibitors of eicosanoid synthesis and their mechanisms of action particularly in inflammation, pregnancy and parturition, ischaemic heart diseases, stroke and hypertension.

2.2.7. Neuropharmacology Research Laboratory (Patch-Clamp Setup)

A state of the art research facility has recently been established at PCMD for conducting neuropharmacology research. The Patch Clamp technique is used for analyzing the drug effects against a series of diseases including cancer, diabetes, depression and dementia. The technique allows high fidelity measurements of small scale ionic currents steering the researchers towards a new era of investigations regarding the role of ion channels in the physiological and pathophysiological functions of excitable tissues thus improving our understanding of a wide variety of disease states with respect to their diagnosis, treatment and prognosis.

2.2.8. General Practical Laboratory

The General Practical Laboratory (GPL) of PCMD is extensively used by students and research scholars of ICCBS. The Laboratory is equipped with many instruments including, Refrigerated Centrifuge, Fume

Hood, Safety Cabinet, Hot Air Oven, Shaking Water Bath, Inverted and Compound Microscopes, Gel Electrophoresis Unit, -20º C and -80º C freezers. Practical sessions during the MPhil courses at the PCMD are also conducted at GPL. These include various techniques in Molecular Medicine including the techniques of Molecular Biology, Biochemistry, Pharmacology, Immunology, Microbiology and Cell Biology.

Figure 14. LC-Mass Spectrometer.

Figure 15. MALDI Tof Tof.

Figure 16. Mass Spectrometer for Electron Impact (EI), Fast Atom Bombardment (FAB) and Chemical Ionization (CI).

2.2.9. The Cell Culture Unit

The Cell Culture Unit is designed and equipped to carry out all general tissue culture protocols. It facilitates the preparation and maintenance of primary isolates, organ cultures, long term cultures as well as cell line based assays. The Unit comprises of three laboratories, each equipped with state-of-the-art cell culture facilities along with additional instrumentation for the processing of cells. This includes Class II Biohazard Safety Cabinets, Carbon dioxide incubators, refrigerated microcentrifuges, liquid nitrogen storage, controlled rate and low temperature freezers and inverted light microscopes equipped with digital cameras. Major activities carried out in the Cell Culture Unit include isolation and propagation of bone marrow stem cells, cardiomyocytes and neuronal cultures, T-cell proliferation and cell-line based cytotoxicity and anti-cancer studies.

2.2.10. Diagnostic Laboratory and Clinical Research Facility

The Diagnostic Laboratory and Clinical Research Facility at PCMD are well equipped with latest and fully automated analyzers. This Laboratory was envisioned to provide highly advanced and rare diagnostic laboratory tests including molecular diagnostic tests which are not commonly available at other laboratories in the country. Equipped with highly advanced, fully automated, computerized technology and staffed by vastly experienced personnel, the Laboratory offers reliable diagnostic testing services with highest level of precision and accuracy. The Laboratory is equipped with state of the art technologies including Flow Cytometer, Cytovision, Gamma Counter, Hematology Analyzer, Hitachi Chemistry Analyzer, Fluorescence microscope and a latest immunoassay system designed to perform special chemistries like, thyroid hormones, insulin, antigen- antibody systems of hepatitis, HIV and other infectious diseases.

2.2.11. Compound Bank

This first Compound Bank of Pakistan was established at PCMD to collect and organize the wealth of natural and synthetic compounds isolated or synthesized at the International Center for Chemical and Biological Sciences. The main objective of the Compound Bank is to support the drug related research at the Center and to co-ordinate various biological assays. The main activities of the Compound Bank include receiving compounds with their complete information, organizing them into various categories depending on source and structural types and arranging them for various types of biological screenings. The data are managed in computer readable form for ready reference.

2.2.12. Animal House Facility

The Animal House Facility (AHF) makes possible the use of animals in research at the PCMD by providing high quality animal care in accordance with the Institutional regulations and guidelines. The ground floor is designated for housing guinea pigs, rats, mice and rabbits. The animal procedure rooms for investigators' use are available in the newly added floor. Apart from these laboratories, space for surgical theatre and post-operative care rooms are also available.

2.3. PLANT BIOTECHNOLOGY UNIT, ICCBS, UNIVERSITY OF KARACHI, KARACHI

Figure 17. Plant Biotechnology Unit, ICCBS, University of Karachi, Karachi**.**

2.3.1. Introduction

The success of modern Plant Biotechnology is attributed to plant tissue culture, which plays very important role in *in-vitro* propagation of disease free plants and multiplying, with an unmatched speed, of endangered species as well as the species difficult to regenerate. The Biotechnology Unit at ICCBS has been working on various aspects to circumvent research to cater the current needs.

2.3.1.1. Commercial Projects

(i) On Pilot Scale:

- Production of virus free banana plants
- Production of different varieties of orchid as a cut flower

(ii) On Experimental Scale:

- Production of cherry tomatoes
- Production of *Anthurium* as a cut flower using Hydroponics System
- Production of *Piper Betel* (*Paan*)

(iii) Plant Varieties Produced through Tissue Culture Technology:

- Orchids 16 Varieties of five Genera
- Banana 12 Varieties; the 5 best are being multiplied by tissue culture techniques
- Croton 20 Varieties (the largest collection in the country)
- *Anthurium* 3 Varieties (high value cut flower in international market)
- Pineapple 2 Varieties , more than 10,000 plants produced and are in the green houses
- Ixora 6 Varieties (A medicinal and ornamental high value plant)
- Dracaena An ornamental high value plant
- African violet An ornamental high value plant
- Fern An exotic ornamental plant having medicinal value
- Furcraea An ornamental and medicinal plant
- Date palm 4 Varieties are under production
- Aloevera Medicinal plant
- Kiwifruit Fruit and a medicinal plant
- Kalanchoe Exotic plant, known as Panda plant
- Ocimum Two species
- Tobacco Medicinal plant
- Tomato Fruit and model
- Potato Stem and model

Figure 18. Patch Clamp for recording of the Current from Single Ion Channel.

Figure 19. Single Crystal X-Ray Diffractrometer for Three Dimensional Structural Analysis of New Small Molecules.

2.3.1.2. Research

The Research Group is comprised of motivated and dedicated students, working towards new perspectives in their particular fields.

(i) Biotransformation: Biotransformation is another important area of Biotechnology to modify the organic compounds of low interest into valuable compounds or to augment the existing efficacy of drugs employing medicinally essential plant cell suspension culture. This is the only lab in Pakistan of its kind.

(ii) Protoplast Hybridization: To develop new traits by combining closely related species having discriminating features and characteristics, protoplast hybridization is applied.

(iii) Genetic Transformation: Genetic transformation is performed to transplant a trans-gene for producing pharmacologically, medicinally, and agriculturally important products.

(iv) Secondary Metabolites Production: This is achieved by using Plant Cell Suspension Cultures.

(v) Tissue Culture Banana Commercialization: The Biotechnology Unit has supported two private labs of the tissue culture in the country, i.e., one in the capital, Islamabad, and the other one in *Tando Alahyaar*, Sindh. Both of the labs are working effectively and providing disease-free banana plants to farmers efficiently.

(vi) Essential Oil Production through Supercritical Fluid Plant: The Biotechnology Unit is also busy in seeking possible application of some of the essential oils in the country; this is right now in developmental phase. The essential oils extracted through supercritical extraction plants system till now are from coriander, cumin, and sesame seeds, and cinnamon. The Unit is exploring feasibility of the above mentioned oil production.

2.4. NATIONAL CENTER OF EXCELLENCE IN MOLECULAR BIOLOGY (NCEMB), UNIVERSITY OF THE PUNJAB, LAHORE

Figure 20. National Center of Excellence in Molecular Biology, University of the Punjab, Lahore.

The National Center of Excellence in Molecular Biology (NCEMB), University of the Punjab, Lahore was founded in 1985 and now comprises state-of-the-art labs and modern equipment for carrying out cutting-edge research in molecular biology and Biotechnology. Apart from its research and library facilities, NCEMB has the land and other requisite facilities for field testing of genetically modified crop varieties. A staff of 160 persons, including 31 senior scientists, 10 Post-doctoral Fellows, 53 MPhil and PhD Research Scholars, nine technicians and 66 administrative and para-scientific staff are employed there.

The Centre has two major research areas, i.e., Health Molecular Biology and Plant Molecular Biology. A brief introduction of each research group and their future plan of work are being presented.

2.4.1. Health Molecular Biology

2.4.1.1. Genetic Diseases Group

Genetic diseases are more prevalent in populations where consanguineous marriages are common. The Group has focused mainly on three areas, i.e., the genetic and molecular basis of Hearing impairment and Vision impairment, which includes Retinitis pigmentosa, Cataract and Glaucoma. Research on Intellectual disability/ mental retardation is recently started. Consanguineous families segregating with these disorders represent a rich genetic resource for the identification of the new loci, genes and pathogenic variants. The prevalence of profound bilateral hearing loss is 1.6 in 1,000 live births in Pakistan. The prevalence of congenital retinitis pigmentosa is 1 in 3,000 cases, 0.3% have congenital cataract and 1-2% cases of intellectual disability have been reported worldwide.

Figure 21. Chromoscan for Karyotyping.

Figure 22. Class II Biological Safety Cabinet.

2.4.1.2. Vision Impairment Group

Identified 550 families with the history of congenital cataract, glaucoma have been ascertained through eye hospitals across Pakistan and discovered 15 novel loci, 12 novel genes and 52 pathogenic variants of known and novel genes for cataract, RP and Glaucoma. The novel findings have been published in *J. Human Genet*., *Am. J. Ophthalmology* and *Molecular Vision*

2.4.1.3. Hearing Impairment Group

Identified 1420 families from different areas of Pakistan and identified 20 new loci, 17 new genes and 75 novel mutations in reported genes. Salient outstanding findings of this Group are the first human *deafness modifier DFNM1* that rescues the deafness phenotype. This was published in *Nature Genetics* (2000). The group has identified a novel tight junction protein that has a critical role in hearing. This work *received Cotterman recognition* and was declared as one of the two best papers published in *Am. J. Hum. Genet*. 79: 1040-51 (2006) and further it was featured in the Editor's choice. Studies on the localization and *function of TRIOBP protein* in hair cell stereocilia was featured in the journal *Cell* 141(5), 2010. Recently the finding that *calcium - and integrin-binding protein* cause Usher syndrome type 1J and nonsyndromic deafness DFNB48 was published in *Nature Genet*. 44: 1265-1271 (2012).

2.4.1.4. Intellectual Disability/Mental Retardation Group

This Research Group has enrolled 250 families residing in different areas of Pakistan and has identified five novel intervals/loci, and 13 reported loci for mental retardation.

The Genetic Disease Group has produced 30 PhD, 58 MPhil students and published 78 research articles in world's renowned journals.

The families having two or more persons with congenital Mental Retardation from different areas of the country will be enrolled. New loci and genes involved in Mental Retardation in such enrolled families will be identified. Further, novel mutations in reported and candidate genes and those affecting the proteins involved in normal brain functions will be identified.

2.4.1.5. Future Direction of Genetic Diseases Group

In view of the above achievements efforts will continue for a sustained search of new and novel genes by combining the classical homozygosity mapping and the newly developed "Targeted Capture and Next Generation Sequencing" methods. The newly discovered genes will be studied for gene function through immune-cytochemistry and cellular localization.

2.4.1.6. Molecular Medicine Group

The Molecular Medicine Group is engaged in the identification of therapeutic potential of medicinal plants against Hepatitis C virus. Toxicological and antiviral analysis was performed on 56 medicinal plant extracts and has identified nine novel plant extracts active against NS3 serine protease of Hepatitis C virus.

Identification, purification and structural elucidation of active compounds from medicinal plants having anti-HCV and anti-breast cancer potential, and the characterization of bacteriophages endolysin gene of Pakistani origin will be performed to test their efficacy against known mastitis pathogen.

2.4.1.7. Bio-Pharmaceutical Group

The Biopharmaceutical Group has developed a state of the art technology to overproduce recombinant human proteins and downstream processing for the purification of therapeutic products. The Group

is working on recombinant human proteins, such as interferons, colony stimulating factors and interleukins. This Group has successfully expressed the human interferon genes in *E. coli* and yeast expression systems. Downstream process for the commercial production of recombinant human interferon has been successfully established. The Group is also working on the pegylation of rhIFN to prolong the persistence of the product in the blood circulation. A Quality Control Lab has been established in the Center to check the activity and maintain quality of the recombinant proteins according to WHO and European pharmacopoeia. This Lab is also providing national facility for bioactivity /potency testing for imported interferons.

2.4.1.8. Virology Research Group

The Group is devoted to study human DNA and RNA viruses prevalent in different geographical regions of Pakistan, their life cycles, structure, attachments, entry to host cell, uncoating, gene expression, replication, and assembly coupled with the development of antiviral vaccines. The Group has developed a PCR based approach and serological assay for the identification of HCV genotypes

Figure 23. Confocal Laser Microscope for the Fluorescence-based Detection of Gene Expression.

Figure 24. Hand held Biolistic Gene Gun for Field and Lab Plant Transformation.

and mixed genotype infections. It has also conducted population based analysis of HCV genotype distribution in Pakistan and rate of viral clearance in patients infected with different HCV genotypes or mixed HCV genotypes.

This Group will develop novel approaches for identification of HCV transmission between hosts using MS profiles of HCV quasispecies and serological profiles of antibody binding to a set of HCV sequence variants of antigens derived from several different HCV trains.

2.4.1.9. Stem Cell Research Group

The Stem Cell Research Group is focusing on exploiting the regeneration potential of stem cells isolated from different adult tissues for repair of seven major organs, namely eye, heart, liver, kidney, cartilage, pancreas and skin.

Ocular repair by amniotic membrane and limbal stem cells is being done clinically in collaboration with Layton Rahmatulla Benevolent Trust (LRBT). The limbal stem cells are cultured from biopsies obtained at LRBT from healthy eye of patients. These stem cells are then transplanted into the damaged eye of the patients for their ocular reconstruction. The patients treated have shown significant improvement in their visual acuity.

Stem cell research on rat/mouse models has demonstrated cardiac repair and improved cardiac function in infarcted as well as diabetes affected hearts. Stem cells in combination with a pharmacological agent also resulted in enhanced recovery of liver fibrosis and improved liver function in drug induced liver fibrosis. Stem cells reduced fibrosis and improved renal function in rat renal failure model.

Bone marrow stem cells were differentiated into Insulin producing cells. The transplantation of these cells reduced blood glucose levels and increased serum insulin levels in diabetic rats. Enhanced wound healing in damaged skin in rats has also been demonstrated by the use of stem cells cultured from adipose as well as skin tissues.

2.4.1.10. Future Direction

The Stem Cell Group aims to expand its collaboration with other hospitals, in addition to LRBT, for providing cultured limbal stem cells for ocular reconstruction in human patients. Also, repair potential of stem cells will be evaluated for the chemically injured skin in rats. Various sources of stem cells will also be exploited for the optimal repair of damaged organs.

The Stem Cell Group has produced 12 PhD, 16 MPhil graduates and published 16 research articles in world's renowned journals.

2.4.2. Plant Molecular Biology

The Plant Molecular Biology is divided into three Groups, namely Plant Transgenic Group, Plant Genomics Group and Seed Biotechnology Group.

2.4.2.1. Plant Transgenic Group

The Plant Transgenic Group is mainly concerned with the Transformation of different genes taken from bacteria and plants The Group has already developed Bt cotton with single and double Bt genes, with and without transit peptide, and Bt herbicide resistant cotton with three genes. For improvement of Physiology, the Group has developed Phytochrome B transgenic Cotton and against sucking insects GNA transgenic cotton has been developed. Currently, the Group is focusing on the development of

CLCV resistant cotton, insect resistant cotton, herbicide resistant cotton, and cotton with all the four desirable traits.

The future research of this Group will include development of colored cotton, along with insect resistance, with a fusion gene under wound inducible promoter, development of transgenic potato/ tomato with interleukin 18 and study of all biosafety concerns of these developed products. This Group has produced nine PhD, 29 MPhil students and published 30 research articles in world's renowned journals.

2.4.2.2. Plant Genomics Group

The Group is working for the identification of novel genes from *desi* cotton (*Gossypium arboreum*) under drought and saline conditions.

The Group has identified drought tolerant genes in *desi* cotton by using microarray and Differential Display. The Essential Sequence Tags (ESTs) have been submitted to the National Center for Biotechnology Information (NCBI). Salt tolerant gene has also been identified and full length gene was isolated and transformed to local American cotton and the transgenic plants were found to be tolerant against salt. Drought tolerant transgenic plants have been tested under greenhouse conditions and have shown 15 days' more tolerance against drought as compared to the non-transgenic plants. A full length gene resistant against CLCV has also been identified in cotton. Presently, the Plant Genomics Group is working for the printing of Oligonucleotide microarray for the identification of drought tolerant genes and cross species analysis of local varieties of cotton model plant, i.e., *Arabidopsis thaliana*. Moreover, identification of drought tolerant genes expression is under investigation in the local cotton varieties. Studies for the CLCV tolerance in cotton are also under way.

In future, the Group is extending its research for the identification of novel genes in cotton and other agriculturally important crops under abiotic stress conditions, i.e., cold, frost, etc.

The Plant Genomic Group has produced six PhD and 10 MPhil students and published 15 research articles in world's renowned journals.

2.4.2.3. Seed Biotechnology Group

The Group is working on biotechnological improvement of seed. It has already developed *Fusarium* resistant Gladiolus by manipulating single cell suspension cultures, which is a very fine tissue culture technique. The NCEMB Hybrid Tomato and Maize have been developed with comparative yield advantage over the commercial hybrids. Similarly, disease free Seed Potato has been produced. This Group has also developed Potato virus Y and Potato leaf roll virus resistant Potato plants along with Sugarcane mosaic virus resistant plants of Sugarcane through the use of RNAi technology. Glyphosate resistant sugarcane has been developed. Currently, the Seed Biotechnology Group is engaged in the transformation of Maize with four gene construct (i.e., Cry1Ac, Cry2A, GTGene and Frost tolerant gene) which render the transgenic plants resistant against insect pests, and tolerant against herbicide and frost. Transformation of Potato with short hairpin RNA is in progress that will render the plants immune against PVX invasion.

A construct is being designed for Potato transformation harboring three short hairpin RNAs, conferring PVY, PLRV and PVX resistance, frost tolerant gene and gene for delay in Potato sweetening.

The Seed Biotechnology Group has produced two PhD, 14 MPhil students and published 33 research articles in world's renowned journals.

Figure 25. Controlled Environment Tunnel for Off Season Plant Growth.

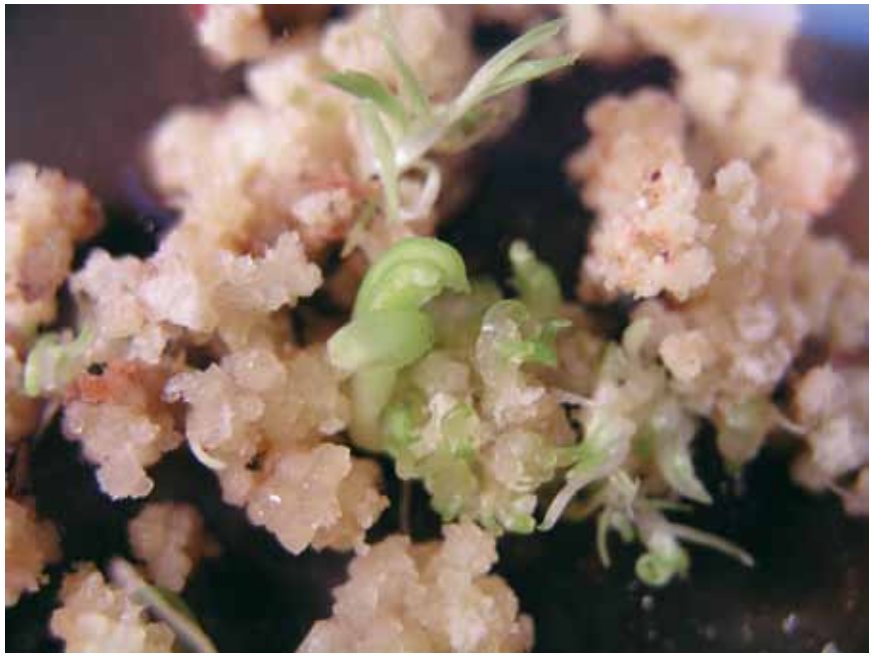

Figure 26. Propagation of Plants through Tissue Culture.

2.4.2.4 Salient Achievements of NCEMB

- Developed Bt transgenic Rce and Cotton, both of which are under field trial.
- Developed Bt pesticide formulation.
- Synthesized four Bt pesticidal genes with optimized codon usage and developed plantexpression vectors containing 2-3 genes.
- Established techniques which are being used to raise disease free seeds of Gladiolus,

Sugarcane, Potato and even fungus free stock of Gladiolus.

- Developed procedures for:
  - o The prenatal diagnosis of β-thalassemia.
  - o DNA Typing for human identification, which is being used in crime investigation and parenthood confirmation.
  - o PCR based diagnosis of Tuberculosis, Hepatitis, SARS, HIV, S. typhi, H. pylori, etc; these services are available to the general public.
- Cloned human pharmaceutical protein genes for commercial production.
- Discovered 11 new deafness loci, 3 new vision impairment loci, 17 new genes and one novel deafness modified gene.
- Approved Patents, 7; under submission Patents, 2.

2.4.2.5. Patents obtained by NCEMB

i) *Bacillus thuringiensis* isolates, active again sucking insects (US Patent No. 6,150,156)
ii) Process development for Bacterial Bioinsecticides (Pak Patent No. 137087)
ii) Process development for Fungal Bioinsecticides (Pak Patent No. 137121)
iv) Methods for Determination of Protein and DNA content for Detection of *Bacillus thuringiensis* in plant products (Pak Patent No. 138279)
v) Development of Bt Basmati Rice Containing Multiple Transgenes (Pak Patent No. 138287)
vi) Molecular Diagnosis (PCR-Based) of Hepatitis C Virus (Pak Patent No. 138719)
vii) Development of modified pegylated recombinant human interferon of therapeutic use (Pak Patent No. 34910035)

2.5. SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF THE PUNJAB, LAHORE

Figure 27. School of Biological Sciences, University of the Punjab, Lahore.

The School of Biological Sciences (SBS), established in July 2002, has made much progress in developing first rate training and research facilities in the field of Molecular Biology and Biotechnology. Currently the school is located in two double storey blocks over a total area of approx. 35,000 sq. ft. Another 3-storey block of laboratories is planned to be constructed to meet the needs of expanding programs of the School. Its laboratories are well equipped with facilities like MALDI TOF-TOF, mass spectrometer,

2-D liquid chromatographic system, capillary electrophoresis, 2-D gel electrophoresis, FPLCs, HPLCs, ultracentrifuge, high speed refrigerated centrifuges, atomic absorption spectrometer and a whole range of equipment of routine use. The School also has specialized facilities like walk-in Cold Room, Animal Cell Culture Laboratory, Animal House, an Insectory and a Green House.

Figure 28. Chemostat for the Production of Biologicals.

Figure 29. Peptide Synthesizer for the Synthesis of Proteins.

The faculty of the School consists of Director General, three Directors, one Professor, one Associate Professor and five Assistant Professors. They are all PhD holders. The faculty of the School is undertaking many funded research projects. Over 80 researchers are working for their PhD. The research is being pursued in different areas, i.e., in agriculture, medical and industrial Biotechnology, proteomics, etc. The specific research projects being undertaken by the faculty are listed below:

2.5.1. Industrial Biotechnology

- Production of bioenergy from plant biomass: Pretreatment of biomass, production of consortium of cellulases and xylanases, saccharification of polysaccharides and alcohol fermentation.
- Cloning and characterization of thermostable enzymes including DNA polymerase and cloning DNA ligase.

Figure 30. An array of Laboratory Fermentors for Biofuel Production.

Figure 31. CD Spectrometer for 2nd Dimensional Structural Analysis.

2.5.2. Medical Biotechnology

- Production and modification of recombinant therapeutic proteins like, insulin, interferon α2b and hepatitis B pre-S protein.
- Preparation of enzymes involved in clinical diagnosis from animal tissues.
- Mutational analysis of genetic modifications in breast cancer, colorectal cancer and leukemia patients.
- Molecular characterization of genetic diseases like dystonia and wolfram syndrome, cleft lip, and moderate to severe hearing loss in Pakistani population.

2.5.3. Agricultural Biotechnology

- Production of growth hormones, preparation of slow-release injectables and their application for enhancing productivity of local farm animals.
- Production and purification of recombinant of insecticidal proteins (Cry1Ac, Cry2Ac and Cry4A) from local isolates of *Bacillus thuringiensis*.
- Molecular and Biochemical characteristics of genes involved in disease resistance in sugarcane.
- Enhancing sugarcane production by modern breeding technology.
- In silico studies on pest translation modifications in transcription factors.
- Identifying the quantitative trait loci associated with salinity tolerance at maturity stage in *Oryza sativa L*.
- Isolation, identification and characterization of plant growth promoting Rhizobacteria from sugarcane.

2.5.4. Others

- Effect of high dose thiamine therapy and molecular aspects of thiamine – dependent enzymes in type2 diabetes patients.
- Study of protein biomarkers for early diagnosis of disease.
- Metallothionein proteins of ciliates.
- Production of recombinant eukaryotic enzymes, like threonine dehydrogenase and 2-amino,3-ketobutyrate CoA, for study of enzyme mechanisms.
- Receptor binding studies of modified bovine growth hormone.
- Identification, characterization and validation of chromosomal markers in Pakistani population for forensic applications.

In addition to the above elaborated Institutes, some new Centers of Biotechnology, namely Dr. A.Q. Khan Institute for Biotechnology and Genetic Engineering (KIBGE) at the campus of University of Karachi, Karachi and Atta-ur-Rehman School of Applied Biosciences (ASAB) at National University of Science and Technology (NUST), Islamabad, have been established. Also, National Institute for Genomics and Applied Biotechnology (NIGAB) has been established at the National Agricultural Research Center (NARC) campus in Islamabad. In KPK, at the campus of University of Agriculture, Peshawar the Institute for Biotechnology and Genetic Engineering (IBGE) is operational

3. HUMAN RESOURCE DEVELOPMENT

For venturing into any Biotech project, it is essential to have appropriate skilled manpower. The Government of Pakistan, with a view to support the knowledge-based economy, established the Higher Education Commission (HEC) in 2002 with the objective of upgrading all the universities to be world class centers of education, research and development. By facilitating this process, the HEC intends to play its part in spearheading a technological revolution in Pakistan.

Since its establishment, the HEC has undertaken a systematic process of implementation of the five-year agenda for reform which includes Access, Quality and Relevance as the key challenges faced by the sector. To address these challenges a comprehensive strategy has been defined that identifies the core strategic aims for reform as: (i) Faculty Development; (ii) Improving Access; (iii) Excellence in Learning and Research; and (iv) Relevance to National Priorities. These strategic aims are supported by well-integrated cross-cutting themes for developing Leadership, Governance and Management, enhancing Quality Assessment and Accreditation and Physical and Technological Infrastructure Development.

Subsequently, HEC embarked on an ambitious program of human resource development by initiating indigenous and foreign PhD programs. The indigenous Master's and PhD programs cover all disciplines of Science and Technology (including Engineering, Basic Sciences, Agriculture and Health Sciences) and Social Sciences and Humanities, although the main emphasis is on Science and Technology. In addition to the indigenous PhD program, the HEC had also initiated a PhD foreign scholarship program of 2000 scholars in which 14 countries are participating. As a result, during 2003-2011, 577 PhDs were produced in Agricultural and Veterinary Sciences and 852 were in Biological and Medical Sciences. Majority of these PhDs are in areas which contribute to the development of modern Biotechnology. This improvement in capacity of the faculty has resulted in an increase in the number of indigenous production of PhDs in our universities. This is further helped by the induction of tenure track system in our academic institutions which ensures market based salaries for the faculty and significant incentives for research.

It is apparent from the data given above that massive efforts have been made to improve the scientific capability, both quantitatively and qualitatively. Most of the scholars in Agriculture, Veterinary, Biological and Medical Sciences are being trained in Biotechnology related areas. In addition, there is a greater emphasis on University-Industry linkages by setting up Technology Parks and Business Incubators at various Universities and R&D Institutes.

It is evident from the R&D activities at the main Biotech Centres and the human resource developed so far, that the major emphasis is on Agricultural and Health Biotechnology. However there are several areas which should be further focused. In case of agriculture**,** major emphasis has been on Bt cotton and on CLCV for obvious reasons. There are many other problems which can be tackled with the help of modern Biotechnology. Some of these are listed below:

- **Cotton**: Insects, virus, drought, salts, fiber quality, weeds, hybrids, etc.
- **Wheat:** Rusts, drought, heat, salts, aphids, weeds
- **Maize:** Shoot borer, high temperature, weeds, hybrids, salts

- **Sugarcane**: Top/stem borer, red rot, rust, mosaic, weeds, low sugar
- **Oilseeds:** High Erucic acid and Glucosinolate
- **Pulses:** Chickpea (pod borer), blight, rust
- **Vegetables:** Fruit fly, mildews, mosaic viruses, blight, worms
- **Fodder crops:** Diseases, pests, weeds, quality
- **Fruits:** Fruit fly, delayed ripening, insects, salinity, drought
- **Others:** Fertilizer Use Efficiency, Biofortification

In the area of health most of the efforts have gone into determining the prevalence of some human diseases, and scoring the type and nature of mutations (both somatic and genetic) in genes directly or indirectly involved in the specific human diseases and genetic disorders, such as partial or complete hearing or vision loss, skin diseases, polydactyly, mental retardation, different types of cancers, etc. In addition, work has been carried out essentially at NIBGE and NCEMB on developing PCR based diagnostic protocols for various diseases such as tuberculosis (TB), Typhoid, Hepatitis B Virus HBV and Hepatitis C Virus (HCV) in additions to some of the cancers. In case of Biologicals, NCEMB and SBS at University of the Punjab have been able to produce Interferon, Human Growth Hormones and Insulin at laboratory scale. Stem Cell Technology has also been developed in laboratories of PCMD, NCEMB and ASAB. However, this technology should be developed as a major research area for specific applications in the field of human health.

4. PHARMACEUTICALS AND BIOLOGICALS

Currently, Pakistan's exports are highly dependent on textile sector. But this area has certain limits because at present 130 countries are producing textiles and clothing but only 30 countries are the buyers. Pakistan urgently needs to diversify its exports and enter into medium to high-tech. industrial sectors, which can only be achieved by human resource development and sensible investment. Pharmaceuticals and chemicals are the two key areas (fall in the range of medium to high-tech.) where Pakistan needs to focus to achieve its future economic goals. Currently, the world medicine market is approximately US$ 550 Billion and Pakistani pharmaceutical market is around US$ 1.3 Billion, with 475 pharmaceutical units registered in the country. Out of these ~94% are local pharmaceutical units whereas ~6% are owned by multinationals but cover 30-40% of the business share.

Local companies are essentially involved in marketing of imported active ingredients and are blending and packing of the finished products for marketing. There is hardly any basic manufacturing and >90% of the cost of the finished product is going to the imported materials. The major problems of low output of local companies are their small size (unable to take R&D initiatives), low capital investment and limited technical skills, lacking suitable infrastructure, low vision of investors and also not having any technical backup support from academia and R&D institutions. To be alive and competitive, every product or technology needs continuous support of locally established R&D, as import of technology (in fragments) is not a long term solution at all.

4.1. HUMAN VACCINES

One area which has been ignored by our academic institutions and R&D institutes is the development of human vaccines. These are the most important public health interventions. Pakistan is almost over 95% dependent on the import of these important healthcare needs. Some of the important vaccines are mainly manufactured in India and in any turbulent geopolitical situation their supply may be threatened. Any potential regional geopolitical change may translate into sanctions like the one imposed on Syria which can lead to shortages of vaccines.

The only public sector manufacturing facility in the country, i.e., Biological Production Division (BPD) at National Institute of Health (NIH), Islamabad, has gradually failed to cater for the ever increasing need. Overall decline of vaccine production at BPD/NIH stem in large part from a long term failure to reinvest in staff, management systems, physical infrastructure, technology, quality assurance and quality control. Any potential outbreak of pandemic flu can overwhelm our health delivery system without having the availability of our own vaccines filling lines.

Thus, the local production of Vaccines and Biologicals is essential for meeting short and long term strategic requirement of Pakistan. This needs to be achieved by building vaccine research and development capability in the public sector and simultaneously attracting private sector to enter into Joint Venture with foreign partners for local production. The Government should support joint ventures which result in complete technology transfer after a certain period of time. The primary market for vaccines is the public sector with main utilization in Expanded Program on Immunization

(EPI), which comprises over 85% of the total vaccine market. The EPI Pakistan spends more than Rs. 1.0 Billion every year on the purchase of BCG, OPV, Measles and TT along with their corresponding numbers of syringes and safety boxes for routine immunization services. Apart from this around Rs. 4.0 Billion are being spent annually to procure oral polio vaccine for special campaign activities in order to achieve the goal of polio eradication.

In order to attract private sector investment the Government of Pakistan will have to provide incentives, such as, buy back guarantee for selected EPI vaccines. Simultaneously, public sector funding for research in this area has to be significantly increased so that effective support to private sector be provided.

4.2. BIOLOGICALS

In addition to its various contributions in the field of vaccines, Biotechnology has played a significant role in the field of biologically active compounds, whose use in medicine is on the increase every day. We see that such compounds are routinely being used in treating various diseases, be it Hepatitis which is treatable with alpha interferon, or be it the conditions where there is a need to boost the body's capability to make blood cells with the help of erythropoietin. Other biologically active compounds, like colony stimulating factor, blood clotting factors, tissue plasminogen activator, growth hormone, insulin, etc., are also on an increasing use in the pharmacopoeia. Pakistan currently is importing all of these biologicals either from the Western world or lately from China and

Iran in addition to a significant amount being smuggled in from India. Thus, we are spending a substantial amount of foreign exchange on these compounds. On top of all this, due to quality control and supply issues, from the latter countries, the quality of these products is very poor; thus, our patients have to suffer needlessly even after wasting a significant amount of their resources by not being cured of the devastating diseases that they are suffering from. So there is a dire need to develop R&D facilities for the production of such biologics and vaccines.

The information about market requirement of some vaccines and biologically active compounds in Pakistan is given in Table 6.

Table 6. Market Requirement of Some Vaccines and Biologically Active Compounds in Pakistan.

Recombinant Proteins	Estimated Consumption in 2002	Estimated Demand in 2010
Hepatitis B Vaccine (Million doses)	20	40
Human Insulin (kg)	150	600
Interferons (Million doses)	1.00	4.00
Streptokinase (Million doses)	0.3	0.9
Erythropoietin (g)	1000	3000

In Pakistan, the carrier rate for hepatitis B is 4-7%; which means Pakistan lies in the intermediate endemicity area. Thus Hepatitis B vaccine has also been introduced in our EPI program. The total requirement for EPI programs are close to 25 Million doses, calculated on the cohort of over five

Million new borns added every year and the wastage factor. The private market is nearly about 02 Million doses. The total existing market is close to 20 Million doses. The Vaccine supplied at EPI is at a cost of 0.3 US$/dose which means 0.3x18 Million is the minimum saving if locally developed vaccine is available. Unfortunately, it is estimated that these requirements will keep growing in the near future. Similarly, development of conjugate vaccines is another area of importance. These vaccines have eradicated pneumonia, meningitis and influenza from the developed countries. Using similar techniques very effective vaccines against diseases like typhoid (the third major cause of child mortality in Pakistan) and dysentery can be developed.

4.3. INCENTIVES FOR THE VACCINE AND BIOLOGICALS INDUSTRY

The Government of Pakistan is requested to consider various incentives to facilitate the development of this sector. These incentives and protection initiatives comprise of the following initiatives in various combinations:

- Tax rebates and special cost sharing in certain components (across the board for the vaccine industry);
- Sharing financial burden of technology transfer rights, royalties, etc. (Vaccine-specific)
- Loan on soft terms;
- Special incentives for export business; and
- Training incentives to technical staff

4.4. VETERINARY VACCINES

Livestock plays an important role in the economy of Pakistan. The entire agriculture sector contributes 21% to the GDP; the share of livestock is more than 50%. It affects the lives of 30–35 Million people in rural areas and is mainly responsible for poverty alleviation. For developing livestock and poultry sector, health coverage and disease control is most important. For this purpose veterinary vaccines play an important role.

4.4.1. Current Status of Vaccine Production in the Veterinary Sector

In the year 2006, six Institutes of the country produced over 32 Million doses of vaccines against five bacterial diseases. Similarly, during the same period the same institutes produced over 282 Million doses of vaccines against nine viral diseases. Despite the aforementioned production, the local requirement of animal vaccines far outstrips the existing production capacity. For more than 105 Million cattle, buffaloes, sheep and goats the country is producing only slightly over 32 doses of vaccines for five different bacterial diseases. It is reported by the Livestock Departments of all Provinces that only 5 to 10% animals are vaccinated each year.

Almost 95% of the vaccines for large animals are produced locally in public sector institutes and only Foot and Mouth Disease (FMD) vaccine is currently imported. However, the coverage of these vaccines is fairly low. Recently, a few private entrepreneurs have also started to produce vaccines but only in a limited quantity. The data from 2006 indicate that over 2-3 Million doses of different vaccines have been produced in the private sector.

The FMD of cattle (a source of great economic loss in the west) is causing heavy losses every year and a large quantity of vaccine against this disease is being imported from France and Iran. There is an urgent need to develop a high quality vaccine against the strains of Foot and Mouth Disease-causing virus prevalent in Pakistan. Development of a DNA Vaccine is most appropriate option.
Domestic Poultry is also not fully vaccinated; however, all commercial poultry farms are 100 percent vaccinated against the viral diseases. The Breeders flocks do not use local vaccine to avoid vertical transmission of virus. It is estimated that poultry vaccines worth Rs 1.2 Billion are imported every year. There is an urgent need to have Specific Pathogen Free Eggs production facilities in the country for the production of better quality vaccines. The Biotechnological Interventions can be very effectively used for production of better quality vaccines for poultry diseases to cater to the ever-increasing demands for all types of poultry vaccines.

All vaccines for the infectious disease of Dogs and Cats are also imported. The Veterinary Research Institute (VRI), Lahore is producing Anti-Rabies vaccine for dogs in a limited quantity but it is not well accepted by the dog owners. They prefer imported vaccines. It is estimated that every year 0.2 Million doses of Rabies vaccine for dogs and cats are imported. To control Rabies, compulsory vaccination of all dogs and cats is required. Biotechnological intervention is essential to produce good quality local vaccine for mass vaccination these animals.

5. REGULATIONS FOR COMMERCIAL EXPLOITATION OF BIOTECHNOLOGY

In the past there have been important impediments in the way of commercial exploitation of the products of Biotechnology. These included a loose and disorganized Intellectual Property Rights (IPR) and non existence of biosafety rules in addition to other allied legislations. During the last few years, such shortcomings have been greatly overcome.

5.1. INTELLECTUAL PROPERTY REGULATIONS

Being a signatory to Trade Related Intellectual Property Rights (TRIPs) Agreement under WTO, Pakistan required upgradation of its intellectual property infrastructure in tandem with the global trends. Accordingly, the existing legislation on Intellectual Property (IP), i.e., Copyrights, Patents and Trademarks, has been upgraded and the revised laws have been promulgated. Earlier all these components of IP were with different Ministries which created a lot of confusion. Now one organization, namely Intellectual Property Organization (IPO; www.ipo.gov.pk), has been established to regulate all matters related to Intellectual Property Rights (IPR).

5.2. BIOSAFETY RULES AND GUIDELINES

Biotechnology applications, specifically in using genetically modified organisms (GMOs), must be accompanied by a systematic risk assessment and management. The Cartegena Protocol on Biosafety (CPB) to the Convention on Biological Diversity (CBD) set the framework on transboundary movement of living modified organisms (LMOs), which required countries ratifying the Protocol, to establish risk assessment and management systems. Pakistan signed and ratified the CBD in 1994 and subsequently also signed CPB in 2001. However, Pakistan took a long time for consultations with all the stakeholders for promulgating the Pakistan Biosafety Rules and Guidelines in 2005 under Section 31 of the Pakistan Environment Protection Act (www.environment.gov.pk/act-rules/Biosafety/Glines.2005.pdf). These rules are applicable to the manufacture, import and storage of microorganisms and recombinant gene technological products for research, for public or private companies involved in the use and application of GMOs and products thereof. These rules are also applicable to field trials of transgenic plants, animals (including poultry and fisheries) and microorganisms.

The Biosafety Rules and Guidelines are comprised of three tiers, namely Institutional Biosafety Committee (IBC), Technical Advisory Committee (TAC) and National Biosafety Committee (NBC). All institutions, both public and private, involved in R&D in Biotechnology are required to establish IBC which is responsible for giving clearance for initiating research according to the Biosafety Guidelines. All applications for approval are initiated by IBC and sent to TAC which comprises of experts and other stakeholders which recommends for approval or otherwise to NBC, which is the final approving authority. Presently, there are more than 20 proposals approved by TAC for trials at different levels. This also includes applications from multi-national companies (MNCs), namely Monsanto and Pioneer Hi-Bred/Dupont, in addition to some national private sector agri-business companies.

Unfortunately presently these Biosafety Rules are in doldrum due to the devolution as a result of 18th Constitutional amendment. Federal Ministry of Environment which was custodian of NBC has been replaced with Ministry of Climate Change. Hopefully these matters are resolved on priority.

5.3. PLANT BREEDERS RIGHT ACT

This Act is to provide an effective intellectual property right system, for granting intellectual property rights to Plant Breeders for the development of new plant varieties. Such an Act is critical to the establishment of a viable seed industry in the country, which is crucial for the development of agriculture. Moreover it is essential for attracting foreign investment into agriculture. The Protection of Plant Breeder's Rights is based on the criteria of novelty, distinctiveness, uniformity, stability and designated by an acceptable denomination of a plant variety. This Act will also facilitate the commercialization of agricultural Biotechnology and will provide incentives to private seed companies. In spite of repeated appeals by all the stakeholders, this Act has not been passed by the Parliament. In absence of this legislation, it is difficult to attract private sector to invest in agribusiness, especially in the seed industry.

6. POTENTIAL BIOTECHNOLOGY PROJECTS FOR PAKISTAN

6.1. AGRICULTURAL BIOTECHNOLOGY

A significant proportion of investment in Biotechnology has gone to agriculture. This has resulted in development of products both from traditional Biotechnology and modern Biotechnology, some which have been commercialized or are ready for marketing.

6.1.1. Traditional Agricultural Biotechnology

6.1.1.1. Biopesticides

The increasing use of chemical pesticides has become a growing concern in recent years, with indications that their use will increase and intensify in the short term. It is therefore an urgent need that we develop and promote the use of alternative methods of crop protection. It is particularly important that efforts be made to substitute chemical pesticides with biopesticides, which are environmentally friendly.

Following are some of the important Biopesticides on which R&D work has been done at various institutes:

- Trichogramma (egg parasitoid) to control lepidopteran pests, such as sugarcane internode borer;
- Fungi (*Trichoderma* and *Gliocladium*) to control root rot and wilt disease in pulses;
- Baculoviruses;
- Nuclear polyhedrosis virus (NPV) of *Heliothis armigera* for cotton, oil-seeds, pulses, vegetables and millets;
- NPV of tobacco caterpillar (*Spodoptera litura*) for tobacco and cotton;
- Granulosis virus (GV) for sugarcane internode borer;
- *Bacillus thuringiensis* for lepidopteran insect control; and
- Neem (*Melia azaderechta):* its various extracts have been marketed.

6.1.1.2. Biofertilizers

The potential of certain microorganisms to improve the availability of nutrients to crop plants has long been known. In view of rise in the cost of chemical fertilizers and their adverse effects on the environment, these microorganisms (collectively called biofertilizers) have become increasingly important. They are considered to be particularly important for soils which are often deficient in organic matter and essential plant nutrients, due to high temperatures and intense microbial activity. Most biofertilizers fix atmospheric nitrogen to ammonia by a complex metabolic process. There are two types of biofertilizer: symbiotic and free-living. The former, which requires symbiotic association with plants, are represented by *Rhizobium*. The latter, which can fix nitrogen independently, include *Azotobacter*, *Azospirillium*, blue-green algae (BGA) and Azolla. In addition several microorganisms from the rhizosphere of various crops have been isolated and characterized which are responsible

for producing growth hormones, mobilize fixed phosphorus in soil and can act as biocontrol agents. Based on such bacteria, NIBGE has produced a biofertilizer which is marketed under the trade name of *BioPower*.

6.1.1.3 Tissue Culture

Modest tissue culture facilities were developed as early as 1968 in the Botany Department of University of Peshawar. There is enormous scope for mass propagation of disease-free plants in several important vegetatively grown crops (like sugarcane, potato, banana, and date-palm). None of these have acquired a commercial level of production, except potato. Potato production with 5% annual growth is a real success story, mainly due to the extensive involvement of the Pak-Swiss Potato Project. This venture has now been taken up by several private firms and non-governmental organizations (NGOs).

6.1.2. Modern Agricultural Biotechnology

6.1.2.1. Molecular Breeding

DNA fingerprinting is a powerful genomic tool that is now widely used in crop breeding programs. This technology is extremely useful in the following areas:

- To evaluate the genetic diversity of a specific crop.
- To determine the relationships among species.
- Identification of molecular markers/ Quantitative Trait Loci (QTLs) for specific traits to launch marker assisted selection (MAS) and construction of genetic linkage maps.
- To isolate genes of interest by making dense genome maps.

In Pakistan, structural and functional genomic tools are being used to assess genetic diversity of crop plants like wheat, cotton, rice and pulses. Research work is being done at NIBGE to find and isolate desirable genes for plant transformation. Such research is also being undertaken by the National Institute for Genomics and Advanced Biotechnology (NIGAB) located at the National Agricultural Research Center, Islamabad.

6.1.2.2. Genetically Modified Crops

In Pakistan, most of the crop improvement activities using modern Biotechnology are focused on cotton, which is among the top five crops of the country. Brassica, chickpea, chilies, cucurbits, potato, sugarcane, tobacco and tomato have recently been taken up. Although transgenic plants of these crops have been obtained but field evaluation was hampered due to delays in approval of Biosafety Guidelines. The Pakistan Biosafety Rules came into force in April 2005, and since then more than 20 applications, from public and private sector, have been filed with the Technical Advisory Committee of National Biosafety Committee for evaluation and approval.

Work on transgenic cotton is at an advanced stage. Some of the national seed companies are also actively involved in multiplication of genetically modified varieties developed at the public sector institutes. In addition, multinational companies, namely Monsanto, Syngenta, Bayer and Pioneer, are also contemplating to market their own products after getting approval from the National Biosafety Committee.

6.2. INDUSTRIAL BIOTECHNOLOGY

6.2.1. Citric Acid

6.2.1.1. Product Overview

At NIBGE, *Aspergillus niger* strains are available and have been extensively studied for production of Citric Acid. The mutant organisms have been developed for application in mass production of Citric Acid. All conditions have been optimized for hyper production of this product. However, further work is needed for mass production of the product. Thus, the main target will be to upscale fermentation process for mass production of citric acid.

6.2.1.2. Market Analysis

Pakistan is importing Citric Acid to meet its demand by spending around Rs. 100 Million per annum. The demand for Citric Acid is on the rise. The indigenous product can substitute or finally replace the imported one. This product will play a vital role in not only reducing foreign exchange spending but will also in giving impetus to the local Biotechnology expertise.

6.1.2.3. Uses and Application

Main use of Citric Acid is in food industry, confectionary and beverages. Approximately 15% of Citric Acid finds usage in chemical industry (removal of sulphur dioxide from flue gases of power stations, metal smelters, metal platting, in detergents, tanning, and textiles) and 10% in pharmaceuticals and cosmetics (solvent and flavoring agent, effervescent with carboric acid, antioxidant and synergist).

6.1.2.4. Raw Material Availability

Main raw material for Citric Acid is sugarcane molasses and beet molasses. These are abundantly and cheaply available. Steep liquor, a byproduct of starch industry, can be used as a nitrogen source.

6.1.2.5. Sources of Plant and Machinery

Ravi Rayon Limited can fabricate the fermenters. Control system (like temperature control, pH control, dissolved oxygen control, on line measuring device for nitrogen source) can be initially imported or fabricated by local industry. Drum filters can be purchased from sugar producing plant fabricators. High-speed centrifuge “Sharples” is available from Alfa Laval, Italy or Sweden and can be used for lactic and acetic acid production as well.

6.2.2. Cellulase

6.2.2.1. Product Overview

The main target will be to upscale fermentation process for mass production of industrial enzymes, initially α-amylase, followed by cellulases, xylanases, proteases and lipases. At SBS,PU, NIBGE and Industrial Biotechnology Institute of GCU, several potent strains are available and have been extensively studied for production of industrial enzymes. Some of the organism produce products with low titres and were improved by mutagenesis or recombinant DNA technology. All conditions have been optimized for hyper production of α-amylase and other enzymes. However, further work is needed for production of other products.

6.2.2.3. Market Analysis

Pakistan is importing about 500 tons of cellulase to meet the demand of around 100 hosiery and denim units in the country at an expense of Rs. 100 Million/annum. The indigenous product can substitute or finally replace the imported one.

6.2.2.4. Use and Application

The main use of cellulase is in textile technology, feed and fruit juice industry.

6.2.2.5 Raw Materials Availability

Main raw materials are wheat straw, wheat bran and other lignocellulosic substrates. These are main agricultural residues and, thus, are abundantly and cheaply available locally. Commercial urea fertilizer, as nitrogen source, and mineral salts are also cheaply available.

6.2.2.6. Sources of Plant and Machinery

Local industry can fabricate the large fermentor vessels. Control system (like temperature control, pH control, dissolved oxygen control, on line measuring device for nitrogen source) can be initially imported or fabricated by local industry. High-speed centrifuge "Sharples" is available from Alfa Laval, Italy or Sweden.

6.2.3. Alpha Amylase

6.2.3.1. Product Overview

Several potent strains are available at NIBGE and have been extensively studied for production of industrial enzymes. Some of the organisms produce products with low titres and were improved by mutagenesis or recombinant DNA technology. All conditions have been optimized for hyper production of α-amylase and other enzymes. The main target will be to upscale fermentation process for mass production of industrial enzymes, initially α-amylase, followed by cellulases, xylanases.

6.2.3.2. Market Analysis

Pakistan is importing about 5000-7000 tons of alpha amylase to meet the demand of 300 textile units in the country by spending Rs. 400-700 Million/annum. The indigenous product can substitute or finally replace the imported one.

6.2.3.3 . Uses and Application

Main uses of alpha amylase are in textile industry, feed industry and production of high fructose syrup.

6.2.3.4. Raw Material Availability

The main raw material for Alpha Amylase is wheat bran, a byproduct of wheat flour mills, and is abundantly and cheaply available. Corn steep liquor, a byproduct of starch industry, is used as a nitrogen source. There will be no scarcity of both the components.

6.2.3.5. Sources of Plant and Machinery

Local industry can fabricate the large fermentor vessels. The control systems (like temperature control, pH control, dissolved oxygen control, on line measuring device for nitrogen source) can be initially imported or fabricated by Pakistan Atomic Energy Commission (PAEC). High-speed centrifuge "Sharples" is available from Alfa Laval, Italy or Sweden.

6.2.4. Lysine

6.2.4.1. Product Overview

At NIBGE, *Corynebacterium glutamicum* strain is available and has been studied for production of Lysine. The mutant organisms will be developed for application in mass production of this product. All conditions will be optimized for hyper production of this product. No the main task is to upscale fermentation process for mass production of Lysine.

6.2.4.2. Market Analysis

Pakistan imports Lysine to meet local demand of animal feed industry. To meet the ever-increasing demand of Lysine, there is a need to indigenously produce the product that can substitute and finally replace the imported one.

6.2.4.3. Uses and Applications

Main use of Lysine is in food industry and animal feed. Lysine is deficient in cereal-based animal feed and needs supplementation by imported Lysine.

6.2.4.4. Raw Material Availability

Main raw material is sugarcane molasses and beet molasses. These are abundantly and cheaply available in the country. Corn steep liquor, a byproduct of starch industry, can be used as a nitrogen source.

6.2.4.. Sources of Plant and Machinery

Ravi Rayon Limited can fabricate the fermentors. Control system (like temperature control, pH control, dissolved oxygen control, on line measuring device for nitrogen source) can be initially imported or fabricated by local industry. High-speed centrifuge "Sharples" is available from Alfa Laval, Italy or Sweden and will be used for lactic acid and acetic acid production.

6.3. HEALTHCARE BIOTECHNOLOGY

6.3.1. Human Healthcare – Vaccine Development

6.3.1.1. Hepatitis B Surface Antigens for the Development of HBV Subunit Vaccine

(i) Product Overview: Hepatitis B virus (HBV) is the most common viral infection in humans all over the world. It is responsible for 80% cases of liver cancer. According to an estimate there are more than 400 Million HBV carriers worldwide with two Million deaths per year. According to WHO, Pakistan is one of the most endemic countries and hence a high risk zone. A project at NCEMB, Lahore aims at producing this medically important vaccine cost-effectively for catering to the local market as well as for the EPI program. It would also help in saving precious foreign exchange spent on the import of expensive vaccines. The vaccine development program of NCEMB will ultimately lead to development of indigenous technology in health and medicine sector. The focus of present studies on HBV at NCEMB is academic that will be helpful in the future production of state of the art technology for the production of Hepatitis B vaccine on commercial scale.

(ii) Market Analysis: Presently, most of the vaccines worth Million of rupees are imported to meet the country's demand. These include Amvax B, Engerix B, Hepatis B, Heppacine B, Hepavax Gene (EPI), Heberbiovac HB, and Heprovac B. Precise data on quantum of imports needs to be gathered to work out the feasibly of the project.

(iii) Uses and Applications: No satisfactory, specific and proven treatment against HBV is available. High cost of the treatment with uncertain results is the major barrier in acquisition of treatment as compared to prevention which is more economical and ensures 95% protection. At present the most efficient method to control HBV infection is vaccination of the new born. In several countries of Asia, including Pakistan, vaccination against HBV has been successfully included in the EPI program.

(iv) Raw Material Availability: The molecular grade chemicals/raw materials required for the production of HBV vaccine are not available locally. These need to be imported.

(v) Sources of Plant and Machinery: The pilot plant facility would require shakers, incubator, fermenter, etc. These are easily available in the international/local market.

6.3.2. Vaccine Development for Egg Drop Syndrome: Oil Emulsion (Poultry Vaccine)

6.3.2.1. Product Overview

Egg Drop Syndrome (EDS) Oil Emulsion Vaccine is effective against the eggs drop syndrome in pullets and layer commercial flocks. It maintains the egg production in layer flock.

6.3.2.2. Market Analysis

The vaccine is not produced locally and has substantial demand by the poultry farmers who are forced to purchase imported vaccine on very high price. If this vaccine could be developed at the local Poultry Vaccine Centres (PVC), it would be made available to the farmers at a much lower price by saving substantial foreign exchange.

6.3.2.3. Uses and Application

This vaccine is used in pullets at the age of 14–18 weeks, preferably before laying. Route of this vaccine is intramuscular or subcutaneous. The use of multivalent vaccines as ND+IB+EDS+ Oil Emulsion is very common.

6.3.2.4. Raw materials Availability

The raw material is available in the local market, except that identification of etiological agents of a local strain needs further research.

6.3.2.5. Sources of Plant and Machinery

The machinery can be fabricated locally.

6.3.3. Research on Human Pharmaceutical Protein

Researchers in medical molecular biology group at NCEMB and School of Biological Sciences at Punjab University, Lahore have cloned several pharmaceutical protein genes which provide an excellent resources material for large scale production of human interferon Alfa 2a, insulin, human albumin, Granulocyte Colony Stimulating Factor (G-CSF), and Erythropoietin. Laboratory scale procedures have

been established and the purity of human interferon Alpha 2a conforms to European Pharmacopeias. However, its large scale production can only be done in a cGMP compliance infrastructure which is still not available in the country.

6.3.4.1. Biopharming for New Drugs and Vaccines

The first recombinant plant-derived pharmaceutical protein (PDP) was human serum albumin, initially produced in 1990 in transgenic tobacco and potato plants. Fifteen years on, the first technical proteins produced in transgenic plants are on the market, and proof of concept has been established for the production of many therapeutic proteins, including antibodies, blood products, cytokines, growth factors, hormones, recombinant enzymes and human and veterinary vaccines. Biopharming is upcoming an area in Pakistan where new drugs and therapeutic vaccines can be developed in plants through genetic engineering. For cost-effective development of therapeutic proteins and edible vaccines, some projects at University of Agriculture, Faisalabad and NIBGE have been launched has been launched based on chloroplast transformation.

6.4. ENVIRONMENTAL BIOTECHNOLOGY

6.4.1. Biocomposting and Generation of Methane from Sugar Mills and Distillery Waste

6.4.1.1. Product Overview

The crystallization of sucrose from sugarcane results into three main byproducts, namely Bagasse, Filter Cake and Molasses. Bagasse is used as fuel in steam boilers, which in turn is converted into mechanical and electrical energy. Filter Cake is the waste of sugar mills. It is produced during the clarification process of sugarcane juices. It is about 3–4% of the total tonnage of sugarcane crushed. The main utility of this waste was to burn it in the brick klins. The disposal of this material remained problematic as it was an environmental issue. The filter contains appreciable macro- and micro-nutrients and organic matter and, thus, is quite beneficial as an organic manure for agricultural purposes. The sugar molasses is the last remain of sugarcane juice from which recovery of sugar is not possible with normal conventional process or it is uneconomical to get more sugar from it. The fermentation of the molasses results into basic organic compound, i.e., Ethyl Alcohol and the waste is also spent wash (stillage). The spent wash is enriched with micro- and macronutrients along with organic matter.

One use of distillery spent wash is to convert it to methane gas through anaerobic fermentation. This gas is desulphurized through chemolithotrophic bacteria and then dehumidified before injecting into generators for producing electricity. The Shakar Ganj Sugar Mills, Jhang and Habib Sugar Mills, Nawabshah have been meeting all their electricity requirements through such process and also are selling the extra electricity to the national grid. There is a great scope for power generation if similar processes are installed in other sugar mills as well. According to an estimate, such processes can generate nearly 1500 MW of electricity.

Figure 32. Biogas Generator.

Figure 33. Methane (Biogas) Holder

Figure 34. H_2S Removal Plant.

Figure 35. Electricity Generator.

6.4.1.2. Market Analysis

No fertilizer with organic matter is available to the growers on commercial scale. In view of increasing cost of chemical fertilizers, the demand for biocompost is on the rise.

6.4.1.3. Uses and Application

The biocompost is a rich source of potash, phosphorus, nitrogen, and micronutrients. It can be used to augment nutrient supply capacity of agricultural fields, to enhance the yield of sugarcane and other crops.

6.4.1.4. Raw Materials

The raw material is available in large quantities as sugar mills in the country have the capacity of crushing more than 45 Million tons of cane annually. In addition, the alcohol industry at present is undergoing expansion and could become a good source of raw material in the form of distillery waste. The sugar mills having distilleries will be able to biocompost the waste material of mills and distilleries.

Wholesale vegetable markets in big cities and towns are another source of raw material in the form of huge quantities of green leaves and vegetable/fruit trash.

6.4.1.5. Source of the Plant and Machinery

The machinery and equipment required for the project is fabricated locally.

6.4.2. Bioethanol

6.4.1.1. Product overview

Ethanol, also known as ethyl alcohol, can be used either as an alternative fuel or as an octane-boosting, pollution-reducing additive to gasoline. Motor fuel grade ethanol (MFGE) is a fast growing market worldwide. Ethanol is also a feedstock for many chemical industries. The USA has already taken lead in bioethanol production over Brazil, and the European Union's output is expected to be more than 3.0 Billion liters per annum. The main raw material for bioethanol is molasses, a byproduct of the sugar industry. Pakistan has nearly 80 sugar mills, out of which 20 mills have distilleries which make commercial grade alcohol (ca. 96% ethanol). Only nine mills make power grade alcohol. Thus, there is a great potential for enhancing this capacity. The Government has already initiated a pilot project for blending gasoline with 10% alcohol (E10). Currently, a major portion of alcohol is being exported.

6.4.1.2. Biofuels from Biomass

With dwindling resources of fossil fuel, the need for production of bioethanol and methane gas from plant biomass is gaining more and more importance. Cellulose and hemicellulose are major components of the plant biomass and these can be degraded to glucose and pentoses, respectively, by the action of a potent and cheap mixture of cellulolytic and xylanolytic enzymes. These enzymes must have the optimal properties of stability, high specific activity and must be available in large quantities cheaply to make the process economically feasible. However, the cost of the enzymes has been a limiting factor in production of biofuels.

Breakdown of cellulose to glucose requires a combination of enzymes, including endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.91) and b-glucosidase (EC 3.2.1.21). The synergistic action of endoglucanases and cellobiohydrolases accounts for most of the cellulase activity; however, β-glucosidase is needed to reduce the inhibitory effects of cellobiose. Similarly, both endo- and exo-activities are required for the breakdown hemicelluloses to pentoses. The laboratories at the School of Biological Sciences of Univeristy of the Punjab, the Industrial Biotechnology Institute of Government College University, Lahore, and National Institute of Biotechnology and Genetic Engineering, Faisalabad have been involved in the production and characterization of microbial cellulases for many years. We are now at a stage that we can apply strategies for over-expression and large-scale production of the selected enzyme activities so that these can be used for the saccharification of celluloses and hemicelluloses of plant biomass. Once this is achieved, the sugars thus produced can be used in a fermentation process to produce alcohol. From laboratory scale and pilot scale experiments, we can then move on to develop processes for large scale production of alcohol.

The breakdown of the plant biomass polysaccharides to sugars, followed by utilization of these sugars by a consortium of anaerobic microorganisms, has also potential for the production of biogas, mainly methane. Studies at NIBGE have produced encouraging results. Further studies would be undertaken to optimize conditions for the various stages of this technology and scaling up the processes ensuring reproducible and consistent outputs.

There are nearly 6.4 Million hectares of salt affected lands. It is proposed to obtain biomass from such areas so that the existing agricultural cropping patterns are not disturbed. Based on this approach, the Government is supporting a pilot project for converting cellulosic biomass to ethanol. The essential steps in this process are as follows:

a) Feedstock washing and milling;
b) Thermo-chemical pretreatment;
c) Enzymatic hydrolysis;
d) Fermentation;
e) Microorganisms seed growth and holding tanks;
f) Ion exchange and chromatographic separations;
g) Distillation; and
h) Solid-liquid separation.

The enzymatic hydrolysis and fermentation equipment includes four 9000-liter, two 1450-liter, and two 160-liter fermenters. All can be used for aerobic or anaerobic fermentation, with separate or combined hydrolysis. The distillation equipment includes a 10-meter stripping column. Majority of the equipment could be fabricated indigenously. However, some equipment for centrifugation and separation could be easily imported.

7. EMERGING TECHNOLOGIES

7.1. RESEARCH ON HUMAN PHARMACEUTICAL PROTEIN

The researchers in Medical Molecular Biology Group at NCEMB and SBS have cloned several pharmaceutical protein genes which provide an excellent resource material for large scale production of human interferon Alfa 2a, insulin, human albumin, Granulocyte Colony Stimulating Factor (G-CSF), and Erythropoietin. Laboratory scale procedures have been established and the purity of human interferon Alpha 2a conforms to European Pharmacopeias. However, cGMP compliance infrastructure is now needed for large scale production of human standards. Such a facility should be developed in the private sector to ensure effective commercialization of the product.

7.2. BIOPHARMING FOR NEW DRUGS AND VACCINES

The first recombinant plant-derived pharmaceutical protein (PDP) was human serum albumin, initially produced in 1990 in transgenic tobacco and potato plants. Fifteen years on, the first technical proteins produced in transgenic plants are in the market, and proof of concept has been established for the production of many therapeutic proteins, including antibodies, blood products, cytokines, growth factors, hormones, recombinant enzymes and human and veterinary vaccines. Biopharming is an upcoming area in Pakistan where new drugs and therapeutic vaccines can be developed in plants through genetic engineering. For cost-effective development of therapeutic proteins and edible vaccines, a project has been launched in Chloroplast Biotechnology Laboratory of Plant Biotechnology Division, NIBGE.

7.3. NANOBIOTECHNOLOGY

Nanotechnology is an art and science of manipulating atoms and molecules to create new systems, materials and devices, with at least one feature having nanoparticles of less than 100 nm. These nanoscale materials have unique chemical and physical properties, much different from their micro- and macroscopic counterparts, which can be exploited for applications in many sectors of the world's economy, including healthcare, consumer products, energy, environment, food and agriculture. For example, surface chemistry of nanoscale materials and devices can be engineered to interact with cells and tissues at a molecular level, for applications in medicine and physiology, with a high degree of functional specificity, thus allowing a better integration between nanotechnology and the biological systems. Similarly, Nature's principles can be exploited to control the assembly of atoms and molecules to fabricate intelligent probes for disease diagnostics and treatment. Moreover, functionalized nanoparticles have recently been used not only to detect a developing tumor at early stages but also its simultaneous treatment. In addition, drugs can be conjugated to the nanoparticles and then injected in the living system to improve their bioavailability and targeted delivery. Due to these and many other potential applications of nanoscale materials in Biotechnology, over 50% of total global funding in the field of nanotechnology is now being directed to explore their applications in Biotechnology, i.e., a field generally known as nanoBiotechnology. Keeping in view the importance of this emerging technology, i.e., nanotechnology/nanoBiotechnology, new undergraduate/graduate degrees and courses are now being introduced worldwide. Our neighboring countries, like China, India

and Iran, have already invested in this technology quite heavily and thus there is a dire need that we should also invest in this emerging technology by appropriate training of manpower and developing institutions with a major focus on nanoscience and technology.

7.4. DRUG DISCOVERY AND STRUCTURAL & COMPUTATIONAL BIOLOGY

At present, drug discovery and structural biology have become an integrated field of research to discover new drugs against various diseases. The field of structural biology is concerned with elucidating molecular function and the basis of biological processes, through analysis of the 3D structures of biomacromolecules and nanobiomachines (proteins) in general and applying that information in particular to the development of pharmaceutical products. The use of structural biology in drug discovery has dramatically changed the way by which new therapeutics are now being discovered. Chemists and structural biologists have been at the forefront of developing new paradigms in drug discovery.

Figure 36. Nuclear Magnetic Resonance (NMR) for Structural Elucidation.

On the other hand, structure-based drug design integrates the use of several computational (homology modelling, docking, etc.), biochemical and biophysical techniques to lead to the discovery of potent inhibitors. Using bioinformatics and high-resolution protein structures, the targets are identified and selected. The identification of potent compounds is made by virtual screening. Structure-based methods are becoming increasingly important in part due to the rapid growth in structural data as a consequence of recent developments in genome sequencing, high-throughput protein expression, purification, crystallization and X-ray diffraction or NMR spectroscopy. Despite these developments, structural biology remains a challenging field to elucidate the structure of many key proteins and protein families. Such approaches for drug discovery using various biotechnological tools have great potential for the pharm industry. It is therefore strongly recommended that strong multidisciplinary expertise be developed in this area.

8. ROLE OF INTERNATIONAL ORGANIZATIONS IN DEVELOPMENT OF BIOTECHNOLOGY

Several International Organizations have been instrumental in the development of Biotechnology in the country. In the early 1980s, USAID provided initial training support by providing Fellowships to our young scientists to get trained and get PhDs in various leading universities in the US. This resulted in a first rate nucleus manpower at the National Institute for Biotechnology and Genetic Engineering, Faisalabad. Several competitive grants were provided through US National Academy of Sciences and US National Science Foundation. Similarly, during that period, JICA (Japanese International Cooperation Agency) established the Plant Genetic Resources Institute (PGRI) at National Agricultural Research Center (NARC) in Islamabad. This Institute is the fore-runner of the newly established National Institute for Genomics and Advanced Biotechnology (NIGAB) at NARC, Islamabad.

During the 1990s, an epidemic of a white fly mediated viral disease occurred in cotton crop which resulted in huge production losses. To overcome this menace, Asian Development Bank provided a loan to the Ministry of Agriculture. A major component of this loan was spent on strengthening Cotton Biotechnology research at NIBGE and NIAB at Faisalabad and NCEMB at Lahore. In addition, a Tripartite Project on Geminivirus – the causative organism of CLCV, was awarded by Common Fund for Commodities (CFC), which is an intergovernmental UN body based in Amsterdam. The project was participated by NIBGE, University of Arizona, USA and John Innes Research Center, Norwitch, UK. It was supervised by the International Cotton Advisory Committee (ICAC), Washington, DC. As a result, these public research institutes were not only able to identify and characterize the cotton leaf curl virus but were also able to develop strategies for imparting resistance to this disease. In the process, an excellent infrastructure for developing transgenic crops was established at atleast two institutes, i.e., NIBGE and NCEMB.

Food and Agriculture Organization (FAO) and World Health Organization (WHO) have been quite supportive of the initiatives of the Government for developing Biotechnology. The WHO established a Molecular Biology Lab at National Institute of Health (NIH) at Islamabad and has been providing research grants in Health Biotechnology through its East Mediterranean Regional Organization (EMRO) headquartered at Cairo, Egypt. Similarly, Human Health Division of International Atomic Energy Agency (IAEA) has been providing technical assistance to develop PCR based diagnostics for infectious diseases.

Presently, there is an ongoing US-Pak Science Cooperation Agreement in which several Biotechnology related projects have been funded. With the help of USDA, two research endowments have been established, one each at Pakistan Agricultural Research Council (PARC) and Pakistan Science Foundation (PSF). Competitive research grants are awarded in collaboration with US scientists. A number of Biotechnology related projects are thus funded. USAID, in collaboration with the Government, has established a Competitive Support Fund (CSF). One of the objectives of this Fund is to establish Business Incubation Centers at the academic institutions so that the technology developed could be transformed into an economically viable venture by developing business plans and conducting feasibility studies. Business incubation is a business support process that accelerates the successful development of start-up and fledgling companies by providing entrepreneurs with an array of targeted resources and services. Therefore, a business incubator's main goal is to produce successful firms that will leave the program financially viable and freestanding. These incubator graduates have the potential to create jobs, revitalize neighborhoods, commercialize new technologies, and strengthen local and national economies The CSF has special interest in supporting commercialization of Biotechnology through this process.

9. JOINT VENTURES

Joint venture in Biotechnology is still at initial stage. There are only a few industries which have entered into joint venture agreements with the foreign companies. In the agriculture sector, Guard Rice, a Lahore based company, has entered into a joint venture with the Chinese Academy of Agricultural Sciences through their authorized agents M/S Biocentury and M/S Silver Land. Some other MNCs, namely Monsanto, Bayer and Pioneer, are trying to market biotech products in Pakistan, especially GM seed, based on the R&D done elsewhere. As stated earlier, all of them will have to get the biosafety clearance.

In the area of pharmaceuticals, biotech products have yet to appear. At the moment, BF-Biosciences have entered into a joint venture agreement with an Argentinean Pharmaceutical Company for production of Interferon and Erythropoitin. Similarly, Getz Pharma has a joint venture with a Dutch company for marketing of pegylated interferon.

The vaccine production industry has also joint ventures with several foreign companies. One of the leading vaccine manufacturing industries is Amson Vaccines and Pharma (Pvt) Ltd. It has joint ventures with Novartis for polio vaccine and with Green Cross Vaccine Corporation for propyhylactic vaccines. Joint ventures with Chinese and Indian companies are also developing.

10. WAY FORWARD AND FUTURE PROSPECTS

Pakistan is a country of nearly 180 Million people. It has basically an agrarian economy with a flourishing service sector giving rise to a sizeable middle class. The agriculture sector, including livestock, has a potential for not only meeting the food requirement but also as an engine for economic growth. For this purpose an input of modern technologies, including Biotechnology, is absolutely essential. In addition, Biotechnology has applications in health, environment and industry. The Government of Pakistan has therefore been liberally investing in capacity building and human resource and infrastructure development for Biotechnology. Pakistan has at the moment a critical number of scientists trained in various disciplines of Biotechnology so that any effort in commercialization could be supported. Also, several indigenous technologies have been developed which could be exploited.

During the last few years, liberalization and deregulation had been the cornerstone of the Government policies. This resulted in many incentives to the entrepreneurs and foreign investors. Recently, many of the MNCs have been showing willingness to invest in this sector, especially in agricultural Biotechnology. In addition, many of the local entrepreneurs are interested to invest and also to enter into joint ventures with the foreign companies. The comparative advantages in such arrangements are that fairly trained scientific manpower and basic infrastructure are available in the country at relatively less cost as compared to the international market.

Since most of the technologies and expertise are with the public sector, it is imperative that a vibrant academia-industry linkage be developed. There is a general lack of entrepreneurship awareness among the academia. It will be worthwhile if an Entrepreneurship Course is made compulsory in our universities. In the absence of such an expertise in Universities and R&D Institutions, there has been a tendency to go for commercialization of their half-baked technologies which has not only brought a bad name but also has proven counterproductive. It is also suggested that the tendency of the academia to go for commercialization on their own should be discouraged, rather it should be made mandatory that collaboration with the private sector be sought. Therefore, it is essential that a clear cut legal framework be developed to encourage academia-industry linkage with transparent SOPs for technology transfer.

Biotechnology is also a main contributor to Small and Medium Enterprise (SME) development by encouraging establishment of Start Up companies. Such an activity can be facilitated by active participation of the Government by providing financial support for such companies because of the absence of Venture Capitalists. In order to achieve this it is suggested that a Venture Capital Fund be established with a contribution by the Government, represented by HEC and MoST, and the private sector, represented by the Federal Chamber of Commerce and Industry. Such a fund would go a long way in commercializing of Biotechnology.

The future of Biotechnology in Pakistan also depends on the active participation of the private sector in commercialization of various indigenously developed technologies and their ability to enter into joint ventures. Moreover, the public sector institutes have to be proactive in transferring the developed technologies to the private sector under transparent agreements. All the enabling regulations, such as IPR and Biosafety rules, are in place, non existence of which was a great hurdle in commercialization in the past.

Another significant step for commercialization of Biotechnology products is establishment of **Biotechnology Parks** at appropriate places. Such facilities should facilitate to upscale the production of various biotech products developed in the laboratories for meeting the need of a bankable feasibility for the potential investor/entrepreneur. Such facilities could also be placed in some of the existing industrial sites.

In order to coordinate the activities related to Biotechnology, a National Commission on Biotechnology (NCB) was established by a notification of the Ministry of Science & Technology (MoST) in 2003. This Commission comprised of eminent scientists and experts from both public and private sector. The NCB acted as an advisory body to MoST to monitor the new developments in the field of Biotechnology at both national and international levels and to recommend appropriate measures. It also made efforts to promote commercialization of Biotechnology products by establishing links between universities, research institutes and industry. The NCB had established its offices in the basement of Pakistan Council for Science and Technology (PCST) building in Islamabad and developed the necessary infrastructure, including an interactive portal. The requisite manpower to run the office was also recruited. All the activities were overseen by an Honorary Chairman and a Secretary who was then Member (Biosciences), PAEC and later Member, Planning Commission. This NCB was being run through a developmental (PSDP) project which finished in 2009, after which NBC's activities could not be continued in spite of several requests made to MoST to give it a permanent status. It is therefore suggested that the Biotechnology Commission may be revived on permanent basis to coordinate all the ongoing R&D related to biotechnology in the country.

Inspite of all these efforts, overall investment on Biotechnology remains sub-critical. This has been further compounded by the current economic crisis resulting in slashing or delaying of a number of Biotechnology related developmental projects. Therefore, at this juncture any additional investment into this sector will be most productive. However, it is necessary to review the status of Biotechnology R&D in the country and its potential for commercialization. It is also worth mentioning that the present Federal Government of Pakistan Muslim League (N) has mentioned Biotechnology and Nanotechnology as the priority areas of Science & Technology in their Manifesto with a plan to establish Foundations for both of these disciplines.

In conclusion, following steps are recommended to facilitate development and commercialization of Biotechnology:

- Liberalization and deregulation of public sector institutions for giving them autonomy to improve their governance;
- Model of Start Up Companies should be followed with a venture-capital type fund to support them;
- Public sector should avoid going into commercial activity by itself; instead, outsourcing or collaboration with private sector is suggested;
- Strong interaction with Chambers of Commerce;
- Model of Public-Private partnership on equitable basis and with foolproof legal protections; and
- Need for Technology Vendor Companies who can do match-making between Academia/R&D Institutions and Industry/Private Sector.

11. SWOT ANALYSIS

11.1. STRENGTHS

Trained scientific manpower, international linkages, strong support through information technology, excellent electronic connectivity, good infrastructure, scientific literature easily available through HEC (more than 2000 scientific journals have been made available online).

11.2. WEAKNESSES

Weak linkages between academia and industry, weak entrepreneurship, bureaucratic hurdles, lack of entrepreneurship among the scientific/academic community.

11.3. OPPORTUNITIES

Deregulated economy, large virgin market with regard to biotech products, increasing middle class, government supportive of private sector, significant incentives for investors, and any initiative will get the status of pioneer industry.

11.4. THREATS

Weak political system, weak governance, law and order situation, current energy crisis.

Appendix 1. Public sector investment in Biotechnology during the last 10 years.

S. #	Name and Location of the Project	Total Cost (Million Rs.)	Sponsoring Agency
1.	Upgradation and Strengthening of National Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad	243.932	Pakistan Atomic Energy Commission (PAEC)
2.	Chloroplast-based Over-expression of Pharmaceuticals to Develop Cost Effective Therapeutics at NIBGE, Faisalabad	35.500	Ministry of Science & Technology (MoST)
3.	Use of RNA Interference Technology for Resistance against Economically Important Gemini-Viruses. at NIBGE, Faisalabad	27.000	Ministry of Science & Technology (MoST)
4.	Development of Nanobiotechnological Research at NIBGE, Faisalabad	155.000	Ministry of Science & Technology (MoST)
5.	Production of Bioenergy from Plant Biomass, by PAEC, PCSIR, University of the Punjab, and GC University, Lahore	295.500	Ministry of Science & Technology (MoST)
6.	Upgradation of National Institute of Food and Agriculture (NIFA), Peshawar	168.240	Pakistan Atomic Energy Commission
7.	DNA Data Bank for Crime Investigation, at CAMB, Lahore	37.900	Ministry of Science & Technology (MoST)
8.	DNA Typing for the Selection of Genetically Superior Cattle and Buffalo, at CAMB, University of the Punjab, Lahore	30.006	Ministry of Science & Technology (MoST)
9.	Disease Resistance through SiRNA Gene Silencing Technique in Local Sugarcane, at National Centre of Excellence in Molecular Biology (NCEMB), Lahore	36.450	Ministry of Science & Technology (MoST)
10.	Cloning and Expression of Selected Human Genes for the Production of the Recombinant Human pharmaceuticals, at CAMB, University of the Punjab, Lahore	37.000	Ministry of Science & Technology (MoST)
11.	National Institute of Genomics & Advanced Bio-technology, (NIGAB), PARC, Islamabad	482.701	Ministry of Food, Agriculture & Livestock (MinFAL)
12.	Development & Commercialization of Biotechnology Products	20.000	Ministry of Science & Technology (MoST)
13.	Development and Promotion of Biogas Technology for Meeting Domestic Fuel Needs of Rural Areas & Production of Bio-Fertilizer.	89.210	Ministry of Science & Technology (MoST)
14.	Development and Application of Plant Tissue Culture Technology for the Production of Stress Tolerant Crops, at PCSIR	15.984	Ministry of Science & Technology (MoST)
15.	Establishment of Centre for Advance Studies in Vaccinology and Biotechnology (CEVAB) at University of Balochistan, Quetta	180.000	Higher Education Commission (HEC)
16.	Establishment of Institute of Industrial Biotechnology, Government College University, Lahore	48.087	Higher Education Commission (HEC)
17.	National Centre for Proteomics, University of Karachi, Karachi (Phase-I)	169.500	Higher Education Commission (HEC)
18.	Establishment of Bioequivalence Centre at Dr. Punjwani Centre, University of Karachi, Karachi	249.130	Higher Education Commission (HEC)
19.	Completion of Ancillary Facilities at A.Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi	37.736	Higher Education Commission (HEC)

(Contd.)

Appendix 1 (Contd.)

S. #	Name and Location of the Project	Total Cost (Million Rs.)	Sponsoring Agency
20.	National Centre for Bio-Informatics, Quaid-i-Azam University, Islamabad (Phase-I)	183.000	Higher Education Commission (HEC)
21.	Study of Protein Biomarkers for Early Diagnosis of Diseases, at School of Biological Sciences, University of the Punjab, Lahore.	31.642	Higher Education Commission (HEC)
22.	Establishment of University Institute of Biochemistry and Biotechnology, PMAS-Arid Agriculture University, Rawalpindi	400.000	Higher Education Commission (HEC)
23.	Strengthening of Institute of Biotechnology & Genetic Engineering (IBGE), KPK Agricultural University, Peshawar	200.000	Higher Education Commission (HEC)
24.	Strengthening of School of Biological Sciences, University of the Punjab, Lahore	400.000	Higher Education Commission (HEC)
25.	HRD and Strengthening of Infrastructure at A. Q Khan Institute of Bio-Technology and Genetic Engineering (KIBGE), University of Karachi, Karachi	475.700	Higher Education Commission (HEC)
26.	Conversion of Traditional Knowledge and Resources into Modern Sciences, Industries and Environmental Protection using Pakistan Indigenous (Plant) Genetic Resources, at University of Karachi, Karachi	38.000	Higher Education Commission (HEC)
27.	Strengthening of National Centre of Excellence in Molecular Biology (NCEMB), University of the Punjab, Lahore	471.134	Higher Education Commission (HEC)
28.	Establishment of Forensic DNA Laboratory at NCEMB, University of the Punjab, Lahore	37.499	Higher Education Commission (HEC)
29.	Establishment of National Centre for STEM Cell and Regenerative Medicine, NCEMB, University of the Punjab, Lahore	384.390	Higher Education Commission (HEC)
30.	Detection of cagA and vacA Genes in Helicobacter Pylori and the Significance of these Genes of H. Plyri in Gastric Cancer and Disease Outcomes, at NCEMB, Lahore	35.000	Higher Education Commission (HEC)
31.	Development of Recombinant Hepatitis B Subunit Vaccine, at NCEMB, Lahore	304.000	Science and Technology Section
32.	Genetic Modification of Rice Plant for Abiotic Stress, at NCEMB, Lahore	36.000	Science and Technology Section
33.	Preparation of Conjugate Vaccine for Salmonella Typhi and Salmonella Paratyphi A., at NIBGE, Faisalabad, in collaboration with Agha Khan University, Karachi	34.000	Science and Technology Section
34.	Upgradation of Human Molecular Genetics Laboratory for the Improvement of Molecular Diagnostics, Therapeutic Research and Establishment of Facilities for Higher Education, at NCEMB, Lahore	36.000	Science and Technology Section
35.	Development and Production of Modern Vaccines, Biologicals and Diagnostic Kits, at NIBGE, Faisalabad	37.434	Science and Technology Section

About the Author

Dr Kauser Abdulla Malik is a Distinguished National Professor of Biotechnology, presently leading Biotechnology Program at Forman Christian College (a Chartered University), Lahore. He is a Fellow of Pakistan Academy of Sciences (PAS) and The World Academy of Sciences (TWAS).

Dr Kauser Malik has been founding Director General of the National Institute for Biotechnology & Genetic Engineering (NIBGE), Faisalabad. He is former Chairman of Pakistan Agricultural Research Council (PARC) (1998-2001); Member, (Bio-Sciences) of Pakistan Atomic Energy Commission (PAEC) (2001-2006); and Member (Food & Agriculture) of Planning Commission of Pakistan (2006-2008). He has been bestowed three civil awards by the Presidents of Pakistan, i.e., *Tamgha-e-Imtiaz,* 1988; *Sitara-e-Imtaz,* 1998; *Hilal-e-Imtiaz,* 2004, for his services to science.

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