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FINAL REPORT

of

symposium-workshop

on

Applications of Molecular Biological Research in
Agriculture, Health and Environment
08-15 April, 1995, Lahore, Pakistan

Project No. GE/GLO/90/004
UNIDO Contract No. 95/078

submitted to

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prepared by

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SUMMARY

The National Centre of Excellence in Molecular Biology, Lahore, Pakistan, under the terms of Contract No.95/078 with United Nations Industrial Development Organization (UNIDO), Vienna, Austria, organized a symposium-workshop on "Applications of Molecular Biological Research in Agriculture, Health and Environment" from 08-15 April, 1995 in Lahore, Pakistan. Twenty-one foreign scientists, one-hundred and thirty scientists from Pakistan and fifteen individuals from local industry attended the symposium-workshop meetings that included sixteen plenary lectures, forty-three short lectures, thirty-five poster presentations, and six workshop discussion sessions.

The symposium-workshop brought together a galaxy of eminent scientists including two Nobel Laureates, who are working to solve specific problems of basic and applied nature in biology, agriculture, and environment through the applications of molecular biological techniques. The symposium presentations specifically addressed latest developments in DNA replication & recombination, genetic restriction & modification, and DNA repair; transgenic plant technology for breeding insect/fungus resistance; medical molecular biology; and environmental biotechnology. The meetings were structured such that an intimate contact between experienced scientists and initiated researchers was ensured. It is anticipated that contacts emanating through the symposium-workshop meetings will lead to joint research endeavours between scientists in the North and the South.

The symposium workshop was preceeded by a two week laboratory training course specifically designed to induct a select group of initiated researchers to molecular biological techniques and concepts that formed the basis of discussions in the symposium-workshop. The two week laboratory training course prepared the participants to receive and assimilate maximum amount of information during the symposium meetings.

Synopsis of all the presentation were collated in the form of an abstract book that was distributed to the participants before the meeting. All plenary lectures were video- and audio- taped for distribution. It is planned to publish the proceedings as a compendium of useful information for active scientists as well as science planners and policy makers.

INTRODUCTION

Biology has been harnessed since antiquity to fulfill humanity's most fundamental needs -- from increasing food supplies to improve health care. Recent discoveries in molecular biological research has made available new and novel methodologies that has expanded the scope of applications. For the first time in the history of mankind, it seems within reach of human endeavours to tailor make plants that can grow normally under stressed conditions, drive off pests, produce unusually high yields, and live for weeks without water. Expression of animal genes in bacteria have enabled production of "wounder drugs" to fight against cancer, AIDs, hepatitis and cardiovascular diseases; synthesis of vaccines to eliminate viral and parasitic diseases; birth of magic prenatal diagnostic procedures for the early detection of human genetic disorders. The vast panorama of applications of molecular biology is just unfolding and it is believed that future uses of novel methodologies will impart new directions to agriculture, environment, industry and medicine. In view of such glowing hopes for doing good and making large profits, it is not surprising that private DNA companies, have proliferated throughout Europe and USA. Most of them have focussed attention on problems in which there are obviously high profits to be made rather than on genuine human needs. In view of such developments taking place at the international scene, United Nations Industrial Development Organization (UNIDO) established an International Centre for Genetic Engineering and Biotechnology (ICGEB), with one component located in Trieste, Italy and the

other in New Delhi, India. Whereas the Italian component has focussed attention on basic molecular biological researches and its medical applications, the New Delhi component has focussed greater attention on agricultural molecular biology. Scientists at both places, are working in the forefront areas to apply innovative biological approaches to solve unique and specific problems of the developing world. Scientists from the developing world come to work in the laboratories of the International Centre to learn specific methodologies and acquire experiences to build local capability. Further, with help and assistance from key Centres in the member states, ICGEB organizes training courses, specialized meetings, symposia and seminars for in depth discussion of local problems and their possible solutions through the innovative applications to latest researches in genetic engineering and molecular biology. Such meetings provide a comprehensive coverage of latest development in selected areas and their possible applications to unique and specific problems of local/regional economic importance.

MAIN BODY OF THE REPORT

As part of five year work programme of ICGEB, covering UNIDO project GE/GLO/90/004, the National Centre of Excellence in Molecular Biology, Lahore, Pakistan received a contract from UNIDO (UNIDO Contract No.95/078) to organize a symposium workshop on "Applications of Molecular Biological Research in Agriculture, Health and Environment" from April 08-15, 1995.

The following areas were selected for discussion during the symposium-workshop meetings:

- DNA replication & recombination
- DNA repair and its relationship to carcinogenesis.
- Genetic restriction & modification.
- Transgenic plant technology with particular reference to breeding insect and fungus resistance
- Medical molecular biology with particular reference to prenatal diagnosis of genetic diseases and production of medically important products.
- Environmental molecular biology with particular reference to bioremediation of soils and underground water.

Participants: Sixteen eminent scientists (Annexure-I) were selected as plenary speakers on the basis of,

- o scientific skills and excellence,
- o relevance of the work done tc the overall objective of the symposium-workshop,
- o their role in a strong university-industry interaction,
- o interest of the individual scientist to promotion of science and technology in a developing country,
- o their willingness to promote the new biotechnology in developing countries.

First announcement of the symposium-workshop was circulated by the symposium organizers as well as by the ICGEB through member country national scientific focal points as an activity of the ICGEB. The response was overwhelming; fifty-seven requests were received from scientists in ICGEB Member/Third World Countries (Annexure-II) and 150 requests from scientists in different universities and R&D organizations in Pakistan. Those interested were invited to send in the abstract of their lecture if they wished to make a presentation or a justification to participate if they wished to make a presentation. The local organizing committee examined these applications and made selections on the basis of the following criteria:

- o relevance of applicants research to the content of the symposium and Inter-alia ability to contribute during group discussions.

- o Quality of work to be presented.
- o Proportionate representation if the applicant was from a developing country/ICGEB member state.

On the basis of the above criteria thirty-four applicants were selected from ICGEB Member/Third World Countries (Annexure-III) and fifty-five applicants from Pakistan (Annexure-IV). All the selected candidates were offered free board and lodge for the duration of the meeting in Lahore. In those exceptional cases where the selectee could not manage travel monies from own sources, the symposium organizers offered to reimburse a maximum of 50% of the cheapest economy air travel between the candidate's home city and Lahore, Pakistan. Since a large number of the selectees from the developing countries could not arrange even one-half of the travel funds, the number of participants dropped to only five (Annexure-V).

Organization of the Symposium: The symposium-workshop extended from April 08-15, 1995.

The symposium meetings were divided into separate sections dealing with basic biology, agriculture, health & medicine and environmental biotechnology (Annexure-VI). The programme of presentation was divided into sixteen 30 minutes plenary lectures by invited experts, forty-three 20 minutes short lectures by the registered participants, and thirty-five poster presentations. The plenary lectures provided a comprehensive review of the presently available information and pointed out problems of future

research. Thus plenary lectures were extremely important and useful for initiated researchers as well as for the scientific policy makers and administrators. The plenary speakers also reviewed literature information for subsequent speakers.

In addition, six concurrent workshop sessions were arranged to provide a rather informal forum at a smaller participation level for the initiated researchers to present and discuss one's own results in an informal atmosphere.

All the plenary lectures were audio- and video-taped. The discussions were duly recorded. It is anticipated that the available information will be used to prepare proceedings of the symposium-workshop as a compendium of useful information.

Two Week Training Course for a Select Group of Initiated Researchers: Immediately preceding the meeting, a two week laboratory training course was organized for 16 young university researchers who were selected to participate in the symposium workshop and who intend to pursue research career in genetic engineering and recombinant DNA technology. The purpose of the laboratory training course was to prepare a group of young scientists so that they could truly benefit from lecture presentations in the symposium, digest and assimilate the lecture content and actively participate in the discussions generated during the meeting. The laboratory course is considered an extremely useful exercise as it helped to impart practical laboratory experience. Needless to mention, this activity was

used to derive maximum benefit from the presence of elite scientists such as R.J. Roberts (Nobel Laureate), H.O. Smith (Nobel Laureate), Stuart Linn, Charles Radding, and Lawrence Grossman.

Workshops: Six concurrent workshops were arranged to provide opportunities to the foreign scientists to obtain first hand information of the existing work conditions, the quality of ongoing research and scientific maturity of scientists in the developing countries. It is anticipated that the proposed activity will help to initiate a meaningful and long lasting international cooperation between the North and the South.

Posters: Thirty-five Posters were presented in the main lobby of the National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore. The poster session was designed to display the results of selected thirty-five scientists. Presentation of poster provided opportunities for discussion of data at a level which provided intimate contact between the presentor and the audience. A committee comprising of Dr. Hamilton O. Smith, Dr. Lawrence Grossman and Dr. Charles Radding evaluated the posters and announced Poster No.26 as the best presentation to win the first prize.

Visit of Invited Scientists to Universities: Two days succeeding the symposium-workshop were devoted to visits of universities and discussions with the faculty and students as well as meetings with government officials involved in R&D planning and policy

making. A different programme of visit was drawn for selected foreign scientists, on the basis of research interest of the department to be visited and that of the guest scientist. The foreign scientists were accompanied by 1 or 2 junior scientists of the Centre who acted as local guides. This activity provided an additional opportunity for interaction between initiated Pakistani scientists and foreign experts and thus helped to develop a rapport that will be used to maintain future communication and/or develop collaborative research endeavors.

Potential Value to Pakistan: Pakistan like all other developing countries needs to strengthen basic and applied molecular biological research as well as relationship between university research and private industry. During the last five years, a number of developments have taken place which are clearly indicative of the increased awareness on the part of both the scientific community and the government about this important linkage. This awareness stems from the promised future applications of genetic engineering made possible only through recent discoveries in basic molecular biological research.

The theme of the symposium-workshop closely relates to the ongoing work programme at the National Agriculture Research Centre (NARC), Islamabad; Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad; National Institute of Genetic Engineering and Biotechnology (NIBGE), Faisalabad; A.Q. Khan's Biomedical Research Laboratories, Islamabad; National Centre of Excellence in Molecular Biology, Lahore. Pakistani laboratories are

working in related areas and therefore the laboratory workers were able to make presentations during the symposium meetings as well as workshop discussions. The symposium, therefore, provided them a forum for discussion with invited experts at both formal and informal levels. The opportunity was usefully used to **a)** introduce the present activities and future programmes of the different research institutions in Pakistan to scientists from the North and the South, **b)** formulate directions for future research on the basis of discussions generated during the symposium-workshop and **c)** to develop contacts which may be used to develop collaborative research programmes involving institutions in the South with those of the invited foreign experts.

TERMINAL SECTION

The National Centre of excellence in molecular biology, Canal Bank Road Thoker Niaz Biag, Lahore, Pakistan organized a symposium-workshop on "Applications of Molecular Biological Research in Agriculture, Health and Environment". The symposium was attended by twenty-one invited scientists from outside of Pakistan, fifty-five participants from different Pakistani universities and R&D organization and seventy-five scientists from local colleges and universities in Lahore. The symposium was divided into different sections dealing with sixteen plenary lectures and forty-three short presentations. In additions, thirty-five posters were displayed to highlight the work of the laboratories of the participants.

The presentations made in the symposium meetings gave a comprehensive coverage to DNA replication & recombination; DNA repair and carcinogenesis; genetic restriction & modification; prenatal diagnosis of genetic diseases; production of diagnostic biochemicals; transgenic plant technology with particular emphasis on the entry and expression of Bt pesticidal genes and anti-fungal proteins.

Abstracts of all the presentations were solicited before the meeting and collated in the form of an Abstract Book (Annexure-VII) which was distributed to all the registrants of the meeting. The abstract book provided recorded information of the various presentations. It is planned to publish the proceedings that will serve as educational material for the entire scientific community.

ANNEXURE-I

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Mr. Carlos Serrano,
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Dr. Mustafiz-ur-Rehman,
Department of Biochemistry,
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Prof. Samir Bejar,
Dircteur de l'Unite SUCRE,
Secretariat D'Etata la Recherche
Scientifique, Centre Dc Biotechnologie,
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ANNEXURE-IV

LIST OF APPLICANTS FROM PAKISTAN

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Pakistan Council of Scientific and
Industrial Research (PCSIR),
16, Sector H-9,
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Senior Scientific Officer,
Plant Pathology,
Cotton Research Institute,
Sarkand.

Dr. Muhammad Ashraf,
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Department of Chemistry,
Islamia University,
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Dr. Faiz-ul-Hussan Nasim
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Department of Pharmacy,
Islamia University,
Bahawalpur.

Dr. Iqrar Ahmed Khan,
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University of Agriculture,
Faisalabad.

Dr. Khalida Sultana,
Department of Biological Sciences,
Quaid-e-Azam University,
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Dr. Abdul Latif,
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Allama Iqbal Medical College (AIMC),
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Lahore.

Ms. Anjum Ara
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Dr. Ikram-ul-Haq
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Qazi Mahmood -ul-Hassan
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Shahid Iqbal
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NODP, Lahore.

Dr. Sarwar Alam,
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Rawalpindi.

Ms. Sumbal Mahmud
INMOL, Lahore.

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Dr. Shagufta Khaliq,
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ANNEXURE-V

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Dr. Mustafeez-ur-Rehman,
Department of Biochemistry,
Dhaka University,
Dhaka-1000,
Bangladesh.

ANNEXURE-VI

PROGRAMME

for

FOURTH INTERNATIONAL SYMPOSIUM WORKSHOP

on the

APPLICATIONS OF MOLECULAR BIOLOGICAL RESEARCH IN AGRICULTURE, HEALTH AND ENVIRONMENT

SATURDAY, APRIL 08, 1995

1800 - 2100 Hrs	GET TOGETHER/REGISTRATION

SUNDAY, APRIL 09, 1995

0800 - 0900 Hrs	REGISTRATION OF PARTICIPANTS (Continued)
0900 - 1030 Hrs	INAUGURATION
o	NATIONAL ANTHEM
o	RECITATION FROM THE HOLY QURAN
o	INTRODUCTION TO SYMPOSIUM WORKSHOP by Dr. S. Riazuddin, Professor & Director, CEMB, Lahore.
o	MESSAGE by L. Grossman, Chairman Foreign Organizing Committee
o	WELCOME ADDRESS by Lt. Gen (Rtd) Mohammad Safdar, Vice-Chancellor, University of the Punjab, Lahore, Pakistan
o	INAUGURAL ADDRESS by the Chief Guest
o	REFRESHMENT

SESSION I

BASIC AND MEDICAL MOLECULAR BIOLOGY

1030 - 1100 Hrs	DNA METHYLASES
	Prof. R.J. Roberts (Nobel Laureate), New England Biolabs, USA.
1100 -1130 Hrs	INITIATION OF CHROMOSOMAL DNA REPLICATION
	R. McMacken. Johns Hopkins University, Baltimore, USA.
1130 - 1200 Hrs	THE FIDELITY OF DNA REPLICATION
	L. Loeb, University of Washington, Seattle, USA.

1200 - 1230 Hrs	MOLECULAR MECHANISMS OF GENETIC RECOMBINATION
	C. Radding, Yale University, Sch. Med, Connecticut, USA.
1230 - 1330 Hrs	LUNCH

SESSION II

***BASIC AND MEDICAL MOLECULAR BIOLOGY** (continued)*

1330 - 1400 Hrs	MOLECULAR BASIS OF GENETIC TRANSFORMATION IN BACTERIA
	H.O. Smith (Nobel Laureate), Johns Hopkins University, Baltimore, USA.
1400 - 1430 Hrs	REPAIR OF UV IRRADIATED DNA IN PROKARYOTES.
	L. Grossman, Johns Hopkins University, Baltimore, USA.
1430 - 1500 Hrs	MISMATCH REPAIR AND GENETIC STABILITY
	P. Modrich, Duke University, Medical Center, Durham, USA.
1500 - 1530 Hrs	PROPERTIES OF HUMAN DNA POLYMERASES
	Stuart Linn, University of California, Berkeley, USA
1530 - 1600 Hrs	TEA
1600 - 1830 Hrs	DISCUSSIONS

MONDAY, APRIL 10, 1995

SESSION III

PLANT MOLECULAR BIOLOGY

0800 - 0830 Hrs	CROP PRODUCTION IN THE DEVELOPING WORLD AND CROP IMPROVEMENT STRATEGIES
	Dr. Gurdev Khush, International Rice Research Institute. Manila, Philippines.
0830 - 0900 Hrs	CROP YIELD SCENARIO IN PAKISTAN
	S.H. Mujtaba Naqvi, NIAB, Faisalabad.
0900 - 0930 Hrs	CROP LOSSES AND CONTROL MEASURES
	Dr. Zahoor Ahmad, Central Cotton Research Institute, Multan.
0930 - 1000 Hrs	IMPACT OF TRANSGENIC TECHNOLOGY ON AGRICULTURE OF PAKISTAN -- A FOCUS ON DEVELOPMENT OF VIRUS RESISTANT COTTON
	Dr. Kauser A. Malik, National Institute for Biotech. & Genetic Engi. Faisalabad.
1000 - 1030 Hrs	TEA

SESSION IV

PLANT MOLECULAR BIOLOGY (continued)

1030 - 1100 Hrs	BT AN ENVIRONMENTALLY FRIENDLY AGENT FOR CONTROLLING PLANT PESTS. S. Riazuddin, Centre of Excellence in Molecular Biology, Lahore, Pakistan.
1100 - 1130 Hrs	PLANT-CODON-OPTIMIZED BT GENES IN RICE Illimar Altosaar, University of Ottawa, Canada.
1130 - 1200 Hrs	INDUCTION OF DEFENSE RESPONSES IN RICE Jan E. Leach, Department of Plant Pathology, Kansas State University, USA.
1200 - 1230 Hrs	CHITINASES AS PROTECTIVE AGENT IN PLANTS M.P. Gordon, University of Washington, Seattle, USA.
1230 - 1400 Hrs	LUNCH
1400 - 1600 Hrs	DISCUSSIONS

TUESDAY, APRIL 11, 1995

SESSION V

0900 - 1300 Hrs	POSTER-VIEWING
1300 - 1400 Hrs	LUNCH

CONCURRENT SESSIONS *(1400 - 1700 Hrs)*

SESSION VI	***DNA REPLICATION, RECOMBINATION, RESTRICTION & MODIFICATION AND REPAIR***
SESSION VII	***GENE CLONING AND TRANSGENIC TECHNOLOGY***
SESSION VIII	***PLANT TRANSFORMATION***
SESSION IX	***MOLECULAR DIAGNOSTICS AND GENETIC DISEASES***
SESSION X	***FUNGAL PLANT INTERACTION***
SESSION XI	***ENVIRONMENTAL BIOTECHNOLOGY***
1700 - 1830 Hrs	CONCURRENT WORKSHOP DISCUSSIONS
1830 - 1900 Hrs	BREAK
1900 - 2000 Hrs	**CONCLUDING SESSION**
2000 - 2200 Hrs	**SYMPOSIUM DINNER (By Invitation)**

WEDNESDAY, APRIL 12, 1995

0900 - 1200 Hrs	VISIT TO UNIVERSITY OF THE PUNJAB, AND MEETING WITH FACULTY
1400 - 1500 Hrs	DISCUSSIONS

THURSDAY, APRIL 13, 1995

VISIT TO QUAID-E-AZAM UNIVERSITY, ISLAMABAD AND MEETING WITH FACULTY

FRIDAY, APRIL 14, 1995

SIGHTSEEING

SATURDAY, APRIL 15, 1995

DEPARTURE

Note-1:	***SESSION I-IV WERE HELD AT SUTLEJ HALL, AVARI RENAISSANCE***
Note-2:	***SESSION V-XI WERE HELD AT CEMB COMPLEX***

ABSTRACTS

FOURTH INTERNATIONAL SYMPOSIUM-WORKSHOP ON APPLICATIONS OF MOLECULAR BIOLOGICAL RESEARCH IN AGRICULTURE, HEALTH AND ENVIRONMENT

APRIL 08-15, 1995

Centre of Excellence in Molecular Biology
University of the Punjab
Thokar Niaz Baig
Canal Bank Road
Lahore-53700
Pakistan

PROGRAMME

for

FOURTH INTERNATIONAL SYMPOSIUM WORKSHOP

on the

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o	MESSAGE by L. Grossman, Chairman Foreign Organizing Committee
o	WELCOME ADDRESS by Lt. Gen (Rtd) Mohammad Safdar, Vice-Chancellor, University of the Punjab, Lahore, Pakistan
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	R. McMacken. Johns Hopkins University, Baltimore, USA.
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	Dr. Kauser A. Malik, National Institute for Biotech. & Genetic Engi. Faisalabad.
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PLANT MOLECULAR BIOLOGY (continued)

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Note-1: *SESSION I-IV WILL BE HELD AT SUTLEJ HALL, AVARI RENAISSANCE*

Note-2: *SESSION V-XI WILL BE HELD AT CEMB COMPLEX*

CONTENTS

ABSTRACTS ARE ARRANGED IN ACCORDANCE WITH THE PROGRAMME

POSTER SESSION

POSTER NUMBER	N A M E
1	Ms. Farzana Khanum
2	Ch. Zahid Mukhtar
3	Mr. Nayyar Iqbal
4	Ms. Tahira Malik
5	Mr. Nasir Ahmad Saeed
6	Mr. Idrees A. Nasir
7	Mr. S. H. Qazi
8	Dr. M. Ashraf
9	Ms. Esther Khan
10	Ms. Rahat Makhdoom
11	Mr. Shahid Karim
12	Ms. Roohi Mushtaq et al.
13	Ms. Roohi Mushtaq
14	Ms. Shahnaz Choudhry
15	Dr. Shahida Husnain
16	Mr. M. Sohail
17	Mr. Farrukh Alvi
18	Dr. Faiz-ul-Hussan Naaim
19	Dr. Khalida Sultana
20	Mrs. Shaheen N. Khan
21	Mr. Z.Q. Samra
22	Ms. Rubina Tabassum
23	Mr. Kalim Tahir
24	Dr. M. Amjad
25	Dr. Bushra Chaudhry
26	Ms. Shahina Maqbool
27	Ms. Zakia Latif
28	Ms. Ghazala Rubi
29	Ms. Anjum Ara
30	Mr. Tariq Javed
31	Dr. M. Ashraf Javed
32	Ms. Sajida Rashid
33	Mr. Abdul Wahid
34	Ms. Kausar Malik
35	Ms. Shaheen Asad

SESSION VI

DNA REPLICATION, RECOMBINATION, RESTRICTION, MODIFICATION AND REPAIR.	
Chairman:	Dr. Amin Hussain
Panel Members:	Prof. M. Anwar Waqar
	Dr. Paul Modrich
	Dr. Stuart Linn
Co-ordinator:	Ms.Roohi Mushtaq
Speakers:	Dr. A. R. Shakoori
	Dr. Faiz-ul-Hasan Nasim
	Dr. Ajaz Rasool
	Ms. Nusrat Jamil
	Ms. Anjum N. Sabri
	Ms. Roohi Mushtaq
	Ms. Shahnaz Choudhry
	Mr. Farrukh Alvi

SESSION VII

GENE CLONING AND TRANSGENIC TECHNOLOGY	
Chairman:	Dr. Mustafizur Rehman
Panel Members:	Dr. Shafqat Farooq
	Dr. Khushnood Siddiqui
Co-ordinator:	Ms. Esther Khan
Speakers:	Dr. Kushnood Siddiqui
	Dr. Shafqat Farooq
	Dr. Mustafizur Rehman
	Ms. Esther Khan
	Ms. Rahat Makhdoom
	Mr. Shahid Karim
	Mr. Nayyer Iqbal
	Mr. Shahid Mansoor
	Mr. Javed Iqbal

SESSION VIII

PLANT TRANSFORMATION	
Chairman:	Dr. M. Akbar
Panel Members:	Dr. Zahoor Ahmad
	Dr. Gurdev Khush
	Dr M. P. Gordon
Co-ordinator:	Dr. Tayyab Husnain
Speakers:	Dr. Iqrar Ahmad/Dr. Azra Qureshi
	Dr. Tayyab Husnain
	Ms. Tahira Malik
	Mr. Aftab Bashir
	Ch. Zahid Mukhtar
	Mr. Nasir Ahmad Saeed

SESSION IX

MOLECULAR DIAGNOSTICS AND GENETIC DISEASES	
Chairman:	Gen. M.A.Z. Mohydin
Panel Members:	Dr. Khalil-ur-Rehman
	Dr. Nasreen Moazami
	Dr. Mohamed Al-Rubeai
Co-ordinator:	Dr. M. Amjad
Speakers:	Dr. Mohamed Al-Rubeai
	Dr. Khalil-ur-Rehman
	Dr. Nasreen Moazami
	Ms. Shaheen N. Khan
	Dr. Shahana U. Kazmi
	Mr. Z.Q. Samra
	Ms. Rubina Tabassum
	Ms. Farah Younus
	Ms. Shagufta Khaliq
	Ms. Anjum Ara
	Dr. M. Amjad

SESSION X

FUNGAL PLANT INTERACTION	
Chairman:	Dr. Azra Sultana Ahmad
Panel Members:	Dr. Farhat Jameel
	Dr. Jan E. Leach
Co-ordinator:	Dr. Bushra Chaudhry
Speakers:	Dr. Tariq Butt
	Dr. Bushra Chaudhry
	Ms. Shahina Maqbool
	Ms. Zakia Latif
	Dr. Farhat Jameel

SESSION XI

ENVIRONMENTAL BIOTECHNOLOGY	
Chairman:	Dr. Anwar Nasim
Panel Members:	Dr Rehan Siddiqui
Co-ordinator:	Dr. Zahoor Ahmad
Speakers:	Dr. Zahoor Ahmad/Sajida Idrees
	Mr. Qaisar M. Khan
	Mr. Abdul Wahid

1

DNA METHYLASES. **R. J. Roberts.** New England Biolabs. Inc., Beverly, USA.

The crystal structure has been determined at 2.8 A^{O} resolution for a chemically-trapped covalent reaction intermediate between the HhaI DNA cytosine-5-methyltransferase, S-adenosyl-L-homocysteine, and a duplex 13 mer DNA oligonucleotide containing methylated 5-fluorocytosine as its target. The DNA is located in a cleft between the two domains of the protein and has the characteristic conformation of B-form DNA, except for a disrupted G-C base pair that contains the target cytosine. The cytosine residue has swung completely out of the DNA helix and is positioned in the active site, which itself has undergone a large conformational change. The DNA is contacted from both the major and the minor grooves, but almost all base-specific interactions betweeen the enzyme and the recognition bases occur in the major groove, through two glycine-rich loops from the small domain. The structure suggests how the active nucleophile reaches its target, directly supports the proposed mechanism for cytosine-5 DNA methylation, and illustrates a novel mode of sequence-specific DNA recognition.

2

INITIATION OF CHROMOSOMAL DNA REPLICATION. **R. McMacken.** The Johns Hopkins University, Baltimore, USA.

Very little is known about the initiation of replication and transcription processes. In our lab, we are using bacteriophage lambda as a model system to study the replication process. Replication is a complex process requiring many protein products. Two phage encoded proteins O and P direct the assembly of the replication fork. The replication fork of lambda is very similar to the *E. coli* replication fork and involves a series of proteins which are the DNA B helicase, DNA G primase, single strand DNA binding protein and a complex DNA polymerase. However, initiation in lambda is unique and for it are required three *E. coli* proteins which are members of the *E. coli* heat shock family. These proteins have been characterized as molecular chaparones and are involved in the rearrangement of nucleoprotein structures at the lambda origin during the initiation process. The ten required proteins have all been cloned and purified. We have established in our lab. a purified protein system that would replicate small supercoiled templates that contain the lambda replication origin. Data will be presented to define how the process of initiation of replication actually occurs and how do different proteins work together to carry out this really complex task.

3

FIDELITY OF DNA REPLICATION L. Loeb. Department of Pathology and Biochemistry, University of Washington, Seattle, Washington, 98195, USA.

DNA replication in animal cells is an exceptionally accurate process. High accuracy is required to ensure that the correct sequence of nucleotides is perpetuated from generation to generation. Yet, some limited variation from parental nucleotide sequences is also necessary for evolutionary adaptation to environmental change. Based upon measurements of spontaneous mutation frequencies in human cells it can be inferred that less than one error is produced for every 10^{10} nucleotides copied. The frequency of errors in the DNA replication process itself could be even lower, since many mutations result from other causes, such as damage to DNA by environmental agents and endogenous reactive metabolites.

In bacteria, mutant DNA polymerases have provided a powerful tool to establish the function of these enzymes and their contribution to the fidelity of DNA synthesis. Few mutant mammalian DNA polymerases are available. We have reported that mammalian DNA polymerase Beta can substitute for *E. coli* DNA polymerase I in both DNA replication and in base excision repair (Sweasy and Loeb, JBC., 267: 1407). Pol Beta increases the rate of joining Okazaki fragments in a *E. coli* polA12rec A718 mutant at non-permissive temperature, suggesting that pol Beta-fills in gaps produced during replication of the lagging strand. In addition, the role of Pol I in the initiation DNA replication of the plasmid pBR322 can also be complemented by Pol Beta. We have used this system to identify a series of Pol Beta mutants and to characterize their properties with respect to DNA replication and DNA repair.

4

MOLECULAR MECHANISMS OF GENETIC RECOMBINATION. C. Radding. Yale University, School of Medicine, Connecticut, USA.

Recombination plays several vital roles in the life of a cell, in case of the immunological system it rearranges pieces of DNA in the chromosome; in meiosis homologous genetic recombination guaranties that homologues will be matched to each other and also repair of double strand breaks. In recent years we have come to understand many of the enzymes in *E. coli* that are responsible for maintaining genetic recombination and they are between 12-24 that are known. At the center of action of these various recombination enzymes is an *E. coli* RecA protein. RecA protein will pair a single strand with duplex DNA to form a three stranded structure and then produce from that a new heteroduplex molecule and a displaced strand. RecA protein puts the single stranded DNA into a helical configuration. In the nucleoprotein filament which is formed by RecA protein there are six molecules of RecA protein per turn. There are nearly twice as many nucleotide residues per turn as there are in B-form DNA. The way that this structure works on the current model will be presented.

5

MOLECULAR BASIS OF GENETIC TRANSFORMATION IN BACTERIA. **H. O. Smith.** The Johns Hopkins University, Baltimore, USA.

Bacteria has three different mechanisms for exchanging DNA. Conjugation mediated by plasmids, transduction mediated by a viral phage particle and transformation which in many ways is the simplest conceptually. In a population of bacteria some of the cells act as donors and release their DNA into the medium which is naked DNA and other cells become competent to take up that DNA actively through the cell membrane and recombine it by a RecA mechanism. The biological importance of this is that populations of cells show polymorphism for different genes. There are many types of mutations and by exchanging DNA between the cells and recombining them increases the overall heterogeneity of the genetic population and thus presumably adapt more easily to new positions. In genetic transformation the organism releases DNA out in the medium and there must be some mechanism to get this highly negatively charged molecule as a polymer through the outer membrane and then through the cell wall, through the enzymes and periplasmic space, through the cytoplasmic membrane and finally to the chromosome in order to undergo recombination. The talk will cover some of the molecular events that happen to the DNA on the way in and also the progress in isolating about twenty genes which are directly involved in membrane transport and recombination studies.

6

MOLECULAR INSIGHTS INTO THE ROLE OF TRANSCRIPTION IN NUCLEOTIDE EXCISION REPAIR. L. Grossman, O. Kovalsky, C. Lin and B. Ahn. The Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 20209-2179.

Functionally active repairosomes were isolated during efforts to characterize the structure of *in vivo Escherichia coli* nucleotide excision repair (NER) competent nucleoprotein complexes. The time of the appearance of these proteins and their location arising as a consequence of SOS induction were correlated with excision of the damaged nucleotides. 80% of damaged nucleotides were excised by constitutive Uvr proteins. The remaining damage was belatedly excised by nascent Uvr proteins. During SOS induction the Uvr proteins and damaged DNA are recruited to the inner membrane where a liponucleoprotein complex is formed. At least 17 proteins are recruited to this complex including repair proteins and RNA polymerase necessary for NER. The capacity of these "repairosomes" to carry out nucleotide excision of the UV-induced SOS thymine dimers is rifampicin-sensitive.

Based on reversible cross-linking studies there is a specific physical interaction between UvrA and the Beta-subunit of RNA polymerase requiring SOS. These data suggest the direct coupling of excision repair to transcription. Strand specificity for Uvr A_2 B binding is predetermined by the 5'->3' directionality of the Uvr A_2 B helicase. The UvrABC endonuclease nicks only on the strand opposite to which it binds. The RNAP signals landing sites for Uvr A_2 B binding because of its ability to distort DNA thereby enhancing Uvr A_2B binding by two orders of magnitude.

7

MISMATCH REPAIR AND GENETIC STABILITY. **P. Modrich.** Duke University, Medical Center, Durham, USA.

Mismatch repair is significant in genetic stabilization. The function of mismatch repair is mutation avoidance and the process stabilizes the genome in several ways. In my presentation I will deal with its best understood function and that is its role in correcting DNA biosynthetic errors. We work on both human and *E. coli* cells and both the mechanism and function of mismatch repair is highly conserved in evolution. Since mismatches are comprised of normal lapses in quick bases, the repair systems have to rely on secondary signals in the helix in order to identify the incorrect nucleotides. In *E. coli* the strand signals necessary for processing DNA biosynthetic errors are based on patterns of DNA methylation at GATC sites, modification of the sixth amino group of adenine. This is an enzymatic modification and that means newly synthesized DNA sequences exist in a transiently unmodified form and it is the absence of the methyl group on the newly synthesized DNA that targets this mismatch repair to the daughter DNA strand. The detailed studies of this pathway will be presented.

8

THE ROLE(S) OF THE HUMAN DNA POLYMERASE. **S. Linn**. Div. of Biochemistry & Molecular Biology, Barker Hall, University of California, Berkeley, CA94720-3202.

Human cells have five known DNA polymerases: , , , and Pol is clearly involved in DNA replication by virtue of its primase activity and its requirement for viral DNA replication *in vitro*. Pol has been implicated in base excision DNA repair synthesis by virtue of its ability to fill small gaps *in vitro* and inhibitor studies *in vivo*. Pol is responsible for mitochondrial DNA excision repair. Pol S is implicated in DNA replication through its requirement for viral DNA replication *in vitro*. However, recent work with *in vitro* nucleotide excision repair systems implicate it in DNA nucleotide excision repair. Pol was first isolated as a DNA repair factor for permeabilized human fibroblasts. Extensive purification of HeLa pol yielded a dimer containing a >220kDa catalytic peptide and a tightly-bound, 55-kDa peptide. We have recently prepared monoclonal antibodies against a purified preparation of HeLa pol , and have obtained antibodies recognizing the >220kD catalytic subunit and the tightly bound 55kD subunit as expected. However, antibodies to a 85-kDa peptide, a 70-kDa peptide and to a 70-and 49-kDa peptide pair were also obtained. These peptides are found in side fractions of purified pol . Among the antibodies recognizing the >220-kDa subunit or the 70-and 49-kDa peptide pair are species which can neutralize-and/or immunodeplete the polymerase activity. Microsequencing of the 85-kDa and 70-kDa peptides identified them as the subunits of Ku protein, a DNA binding protein and helicase implicated in recombination, repair and transcription functions. Indeed, the antibodies can recognize Ku subunits expressed in *E. coli* and antibodies to the Ku subunits can immunodeplete the catalytic pol subunit. We have also used the antibodies to show that the same >220-, 70-, and 49-kDa antigens are present in a mammalian protein complex (RC-1) that repairs double-strand breaks and deletions by recombination *in vitro* (Jessberger, *et al.*, 1993, JBC 268, 10570). Moreover, the large form of a mammalian damage DNA binding protein (Keeney, et al. 1993, JBC 268, 21293) contains the >220-,85-,70-, and 47-kDa antigens. All of these observations suggest that Ku protein can form functional complexes with pol which may be involved in recombination and recombinational DNA repair. Finally, recent experiments with the antibodies also suggest a role for pol in replication.

9

CROP PRODUCTION IN THE DEVELOPING WORLD AND CROP IMPROVEMENT STRATEGIES. G. Khush. International Rice Research Institute, Manila, Philippines.

Combination of conventional as well as some recent techniques in Molecular Biology are crucial for crop improvement. There are three most important crops which feed the world which include wheat, rice and maize. Wheat has the highest production, second is rice and third is maize. Most of the increase in food production has been in the cultivated area but in the future most of the increase will need to occur from less land, less water and less labour than what was available in the last 25 years. Crop improvement strategies involving conventional plant breeding, cell and tissue culture, use of molecular markers and genetic engineering will be reviewed.

10

CROP YIELD SCENARIO IN PAKISTAN. M. Naqvi. NIAB, Faisalabad.

Pakistan is a land of immense agricultural potential. In the north the Himalayas, the Hindukush and the Karakorum provide a temperate climate whereas in the South there is semi-arid region. The Indus river provides plentiful water which can be used to grow a range of crops and exotic fruits. However, in order to achieve the desired yields the country faces major constraints which include water shortage, energy shortage, deforestation and salinity. Sun is the major source of energy. For the lack of alternative methods the plants are the best source for tapping solar energy. Agriculture is nothing but harvesting solar energy and therefore plants deserve more emphasis than they receive.

11

AN OVERVIEW OF COTTON PRODUCTION IN PAKISTAN. Z. Ahmad. Central Cotton Research Institute, Multan, Pakistan.

Cotton is ancient to Asia. The name of the Indus Valley is inextricably linked with cotton in both legend and history. Archaeological excavations bear testimony to the fact that oldest cotton yarn and fabric known today, were from the ruins of Mohenjodaro, 320Km, North of Karachi. These articles have been estimated to be about 5000 years old. Very recently cotton seed from the 5th millennium B.C. has been discovered at Mehr Garh in Baluchistan Province of Pakistan.

Cotton is of vital importance to the economy of Pakistan. It is grown over an area of 2.87 million hectares in the Punjab and 0.548 million hectares in Sindh. It is now feeding over 400 units of spinning mills with about 8.1 million spindles, 1035 ginning factories and about 5000 oil expellers. These mills are providing jobs to millions of workers. It is estimated that textile industry is employing over 35% of the industrial labor force and according to the statistics of 1991-92, the export earnings from cotton and its value added goods amount to 68% of the total exports.

In 1991-92 crop season, Pakistan produced record production of 12.8 million bales with a yield of 768Kg of lint/ha. Since then the production has fallen down. In 1992-93 crop season the production dropped to 9.1 million bales with a yield of 543Kgs of lint/ha. In 1993-94 the production further dropped to 7.935 million bales with a yield of 481Kg of lint/ha. In 1994-95, heavy rains and pest attack again affected cotton production. The continuous decline in production made Pakistan an importing country of cotton and lost its continuous status of 2nd largest exporter of lint of cotton in the world. Economy of Pakistan has been affected. The main reasons for the low production of cotton has been untimely rains and floods, attack of Cotton Leaf Curl Virus and out-break of American bollworms (*Heliothis armigera*). These factors will be analyzed.

12

BT AN ENVIRONMENTALLY FRIENDLY AGENT FOR CONTROLLING PLANT PESTS. S. Riazuddin, T. Husnain, E. Khan, R. Makhdcom, F. Khanum, T. Malik, S. Karim, F. Mustafa. Centre of Excellence in Molecular Biology, University of the Punjab, Canal Bank Road, Thokar Niaz Baig, Lahore-53700, Pakistan.

Pakistanian environments were searched for Bt isolates possessing novel entomocidal properties. One thousand isolates were collected and 350 of them characterized for the crystal protein gene content and bioactivity against American bollworm, pink bollworm, spotted bollworm, rice stem borer and rice leaf folder. At least 12 of the isolates show promising entomocidal activities. Three isolates were selected as source material for the isolation and cloning of cryIA(a), cryIA(c) and cryVI genes in plant expression vectors.

Conditions were established for the entry and expression of foreign genes into indica rice Basmati 370 and chickpea vars. 6153 and CM72. The procedures thus established were used to transform rice and chickpea with CryIA(c) genes. Stable entry and expression of CryIA(c) gene in rice and chickpea was demonstrated by Southern and Western blotting methods. The transformants exhibit inherent resistance against target insects.

A byproduct from a local industry was used for the large scale production of an efficacious Bt isolate to develop bioinsecticidal formulation which has been used in field trials. Small scale plot results show that the bioactivity of the local isolate is comparable to Ciba Giegy Agree 0.6 formulation. To delay the development of resistance from the possible use of Bt transgenic plants or Bt bioinsecticide, management strategies will be discussed in relation to data on the mechanisms of action of Bt toxin insect larvae.

13

PLANT-CODON-OPTIMIZED BT GENES [CRYIA(B) AND CRYIA(C)] IN RICE: RAPID ASSAY OF SYNTHETIC GENES BY EXPRESSION IN MAIZE ENDOSPERM-DERIVED CULTURE. **I. Altosaar, R. Sardana, S. Dukiandjiev, M. Giband, X. Cheng, K. Cowan, and C. Sauder**. Department of Biochemistry, University of Ottawa, 40 Marie Curie Private, Ottawa, Ontario KIN 6N5, Canada.

For the ultimate control of yellow stem borer and brown leafhopper larvae in transgenic rice, the synthesis of two genes, CryIA(b) and CryIA(c), each consisting of 1845bp, is described in detail. The genes were synthesized using an improved PCR procedure based on recessive principles. The synthetic CryIA(c) gene was put under the control of a maize ubiquitin promoter. This construct was tested in a maize endosperm-derived suspension culture system. The use of maize endosperm culture as a quick and efficient system to test the activity of synthetic genes will be described.

14

INDUCTION OF DEFENSE RESPONSES IN RICE. J. E. Leach. Department of Plant Pathology, Kansas State University, USA.

A simple model can be derived to explain the single gene concept which is that single genes in plants are responsible for encoding resistance and single genes in bacteria encode a factor recognized by the host plant receptor gene. If the plant has a dominant resistance gene and if the pathogen has a corresponding avirulence gene then you get resistance. In our lab we are trying to understand how resistance is elicited in rice, what are elicitor-receptor interactions which lead to resistance, what is the elicitor doing in resistance response in plants and also what turns on that response in plants to effectively resist the pathogen. The aim of our work is to identify potential genes for resistance so that we might be able to modify the genes that are turned on and are called defense response genes. Peroxidases which invite chitinases as elicitors will be presented.

15

CHITINASES AS PROTECTIVE AGENTS IN PLANTS. **M. P. Gordon.** University of Washington, Seattle, USA.

We have been studying the nature of the wound response by poplars. We have found that, when lower leaves of the poplar are injured, the lower and top leaves respond by selective transcription of a number of genes. The signal follows the phloem and can be correlated with the transport of sugars. Specific wound-inducible genes are transcribed, Multi-gene families termed *win*3, *win*4, *win*6, *win*8 and *chi*X. These appear to code for proteinase inhibitors, for protein related to poplar bark storage protein, and for several families of chitinases. The signal appears to involve methyl jasmonate as an elicitor. Salicylic acid, which is endogenous, and nordihydroguaiaretic acid, which is exogenous, block the transcription of *win* genes. This blockage can be overcome by exogenous application of methyl jasmonate. In poplar trees, the levels of salicylic acid are normally high. Wounding is accompanied by a decrease in the amount of salicylic acid, which appear to remove the block to the action of jasmonic acid.

We have undertaken studies of both *win*3 (proteinase inhibitor) and win6 (chitinase) promoters. These promoters have been utilized in producing transgene constructs, which were then transferred into tobacco plants. The poplar *win*3 and *win*6 promoters were found to function in tobacco plants, and constructs using GUS reporter genes indicated that the *win*3 and *win*6 were expressed in the apical parts of plants, particularly in the axillary tissues of young, developing leaves. In addition, the *win*3-GUS and *win*6-GUS genes were found to be strongly transcribed in ovaries, pollen, and seeds. The time of expression of *win*3-GUS in developing tobacco seeds parallels the expression of *win*3 in the poplar seeds. The possible application of these products in crop protection will be discussed.

16

CYTOKINE-INDUCTION OF PROLIFERATION AND EXPRESSION OF CDC2 AND CYCLIN A IN FDC-P1 MYELOID HEMATOPOIETIC PROGENITOR CELLS: REGULATION OF UBIQUITOUS AND CELL CYCLE DEPENDENT HISTONE GENE TRANSCRIPTION FACTORS. A.R. Shakoori[1], C. Cooper, A. J. Van Wijnen and G. S. Stein. [1]Department of Zoology, University of the Punjab, Lahore, Pakistan. Deparment of Cell Biology AND Hematology & Oncology, University of Massachusetts Medical School & comprehensive Cancer.

To evaluate transcriptional mechanisms during cytokine-induction of myeloid progenitor cell proliferation, we examined the expression and activity of transcription factors which control cell cycle dependent histone genes in IL-3 dependent FDC-P1 cells. Histone genes are transcriptionally upregulated in response to a series of cellular regulatory signals that mediate competency for cell cycle progression at the G1/S phase transition. We therefore focused on factors which are functionally related to activity of the principal cell cycle regulatory element of the histone H^4 promoter: CDC2, cyclin A, as well as RB-and IRF-related proteins. Comparisons were made with activities of ubiquitous transcription or expression of tissue-specific phenotypic properties. Northern blot analysis indicates that cellular levels of cyclin A and CDC2 mRNAs increase when DNA synthesis and H^4 gene expression are initiated, supporting involvement in cell cycle progression. Using gel shift assays, incorporating factor-specific antibody and oligonucleotide competition controls, we define three sequential periods following cytokine stimulation of FDC-P1 cells when selective upregulation of a subset of transcription factors is observed. In the initial period, the levels of SP1 and HiNF-P are moderately elevated, ATF, AP-1, and HiNF-M/IRF-2 are maximal during the second period, while E2F AND HiNF-D, which contain cyclin A as a component, prodominate during the third period coinciding with maximal H^4 gene expression and DAN synthesis. Differential regulation of H^4 gene transcription factors following growth stimulation is consistent with a principal role of histone gene promoter elements in the integration of cues from multiple signalling pathways which control cell cycle induction and progression. Regulation of transcription factors controlling histone gene promoter activity within the context of a staged cascade of responsivenes to cycline and other physiological mediators of proliferation in FDC-P1 cells, provides a paradigm of experimentally addressing interdependent cell cycle and cell growth parameters that are operative in hematopoietic stem cells.

17

IN VIVO SELECTION OF AN OPTIONAL EXON IS AFFECTED BY MUTATIONS IN ITS IMMEDIATE DOWNSTREAM 5' SPLICE SITE. **F. H. Nasim and P. J. Grabowski**[1]. Department of Pharmacy, Islamic University, Bahawalpur-63108, Pakistan, and Department of Biological Sciences, [1]University of Pittsburgh, Pittsburgh, PA 15260, USA.

A number of genetic disorders have been characterized to be the consequences an error in processing of the primary transcript. In eukaryotes, splicing of pre-mRNA molecules is one of the several essential processing steps involved in the maturation of primary messages originating from split genes. Fidelity of selection of an exon, a coding region of the message, during splicing is known to involve a number of cellular factors but the mechanism has not been clearly established. We have shown that the *in vitro* selection of the 3' splice site of an exon is sensitive to the complementarity of its immediate downstream 5' splice site with the 5' end of U1-snRNA (Nasim et al., Genes & Dev., 4:1172-1184, 1990). Based on our *in vitro* studies, we have proposed a U1-snRNP facilitated exon selection model that involves biding of individual U1-snRNPs at the two 5'splice sites surrounding an exon (Grabowski et al., Mol. Cell. Biol., 11:5919-5928, 1991). This model has now been tested *in vivo* using a series of minigenes corresponding to the *in vitro* using a series of minigenes corresponding to the *in vitro* 5' splice site variants of an optional exon E4 from the rat preprotachykinin gene. These minigenes were used to transfect three human cell lines (HeLa, 293, and NMR-32), and the corresponding spliced RNA species were characterized using RT-PCR amplification technique. Although the splicing patterns exhibited in these cell lines were representative of our *in vitro* findings, E4-selection in some of the sequence variants improved significantly *in vivo*. Selection of E4 in the sequence variants with a perfect 5' splice site (UP mutant) or no 5' splice site (DOWN mutant) downstream of E4 showed no change from the corresponding *in vitro* splicing behaviour. However, the sequence variants with a sub-optimal 5' splice site downstream of E4 showed an improved E4-selection, albeit retaining its optional characteristic. Thus, the *in vivo* splicing of an optional exon also appears to follow the two-site U1-binding model, suggesting that selection of an exon in vivo can be modulated by varying the interaction of U1-snRNP with its immediate downstream 5' splice site.

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18

BACTERIOCINS: A UNIQUE CLASS OF PROTEIN ANTIMICROBIALS WITH PROMISING PROSPECTS. S. A. Rasool, A. Iqbal and S. Ahmed. Department of Microbiology. University of Karachi Pakistan.

Bacteriocins are biologically active 'natural' protein antimicrobials (-static/-cidal). They are either posttrans-latioally modified or have an immunity protein component. Bacteriocins are going to prove broad basedly instrumental against the organisms (alone or in combination with other antimicrobials) to maximize the bactericidal activities concomitantly minimizing the adaptation/resistance responses. In addition, bacteriocins like 'nisin' have successfully been used as natural preservatives in order to enhance the shelf life of food; and in health-skin care products (such as toothpaste, mouth-wash, cosmetics and even soaps). One might be optimistic that benefits of bacteriocins may far exceed those currently envisioned in the microbial killing scenario. Laboratory of Molecular Genetics, Department of Microbiology, University of Karachi has been engaged in elucidating the isolation and characterization of bacteriocins from heterogeneous microorganisms. The talk (in addition to fundamental concepts) will be based on interesting findings.

19

AMPLIFICATION OF DIVIVA$^+$ GENE OF BACILLUS SUBTILIS AND ITS EFFECTS ON SPO PHENOTYPE. **A. N. Sabri and S. Husnain.** Department of Botany, University of the Punjab, Lahore-54590, Pakistan.

Hybrid plasmids pSH1012 and pSG1019, which carry divIVA gene of *Bacillus subtilis*, could be integrated in *Bacillus subtilis* chromosome in the region of DNA homology. When transformed in *Bacillus subtilis* divIVA mutant strain (1A196) they yield three types of transformants, i.e., Cm^RSPo^+ (Div^+), Cm^RSPO^--normal (Div^-), Cm^RSPo^--translucent (Div^-). By increasing concentration of chloramphenicol (Cm) 5, 10, 20, 30ug/ml^{-1}) gene amplification of divIVA gene Cm^RSpo^+) was accomplished and its effects on cell and colony morphology (Spo phenotype) was studied on Schaeffer's media. When cultures amplified at higher concentrations were shifted to lower concentrations of Cm the bacterial growth improved. The intensity of brown colour of colonies were more on Cm plates at higher concentrations, viz. sporulation was comparatively more at higher concentrations. Revertants colonies (white, light brown or mixture of white and brown colours) were observed when cultures (Spo^+ transformants) amplified at 5 to 10ug ml^{-1} Cm were shifted to lower concentrations or selection free media. While shifting of amplified cultures from higher concentrations of Cm (20 or 30ug ml^{-1}) to lower concentration/selection free media did not promote the development of revertant colonies. We attribute it to many copies of integrated (divIVA) gene of *B. subtilis*. Cell morphology of revertant colonies revealed that they exhibit cell morphology and sporulation of divIVA mutant and that of dark brown colonies was like wild type *B. subtilis* strain.

20

STUDY OF TYPE II RESTRICTION MODIFICATION SYSTEMS. **R. Mushtaq**. Centre of Excellence in Molecular Biology, University of the Punjab, Canal Bank Road, Lahore-53700, Pakistan.

Research work performed during this study was aimed to discover new type II restriction endonucleases (REases) and clone interesting restriction-modification (R-M) system(s) into *Escherichia coli* to study the expression and primary structure of restricting and modifying proteins. During this study sixty bacterial strains, collected from different ecological environments of Pakistan, were screened for the presence of type II specificity REase. Protein extracts from twenty one strains were found positive for the presence of endonucleolytic activity. Twenty enzymes were proved isoschizomers of already known specificities. One enzyme *Bse*RI isolated from *Bacillus species* R (CAMB 2669) was recognized as a novel specificity (Mushtaq et al., 1993).

Two R-M systems *Ecl*XI, isoschizomer of *Xma*I/*Cfr*9I/*Xcy*I, and *Sau*MI isoschizomer of *Sau*3AI were selected for detailed study.

Bacterial DNA MTases that catalyze the formation of ^{m5}C and ^{m4}C posses related structure. Similar motifs containing invariant position, are found in the same order in almost all the known MTase sequences. On the basis of the above information a strategy was devised to amplify unknown ^{m5}C *sau*MIM gene. Keeping in view the conserved motif **PCQ** (motif IV) and **FNV** (motif VIII) a set of degenerate oligonucleotide primers was designed. The amplified *sau*MIM gene fragment was characterized to contain the **PCQ** (motif IV) and **FNV** (motif VIII) motifs along with the **ENV** (motif VI) motif existing in-between them.

*Ecl*XI R-M system was cloned by shot-gun cloning stratergy into *E. coli*. Six deletion mutants of *RM* genes were partially sequenced and searched for the presence of **SPPY** and **GXG** motifs, characteristic of ^{m4}C genes. There was presence of **TSPPYWGLRD** and **KPISAFGSGDVQI** sequence motifs in the *ecl*XIM gene and **SGCG-HIPHH** sequence motif in the *ecl*XIR gene. Such a sequence **SGTDVSNIHN** for *Xcy*I and **SGNT-TNIGS** for *Eco*RV REases has also been reported by Withers et al. (1992).

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21

SEARCH FOR NEW TYPE II RESTRICTION ENDONUCLEASES FROM LOCAL BACTERIA. S. Choudhry. National Centre of Excellence in Molecular Biology, University of the Punjab, canal bank road, Lahore-53700, Pakistan.

Search for the presence of new type II restriction endonucleases has been carried out from 52 bacterial isolates. 18 are reported positive for the type II enzyme activity. Eight isolates namely, *Bacillus thuringiensis* D_4, *Bacillus schlegellii* S_3, *Bacillus* species 3T, *Bacillus* species CL14.6, *Streptomyces aureomonopodiales*, *Arthrobacter picolinophilus*, *Pseudomonas aeruginosa* Q_2 and an unidentified bacterium PS5(2), contain *Bth*DI (*Bst*NI), *Bsc*AI (*Sfa*NI) *Bsp*TI (*Ava*II), *Bsp*14.6I (*Bst*NI), *Sar*I (*Stu*I), *Api*I (*Pst*I), *Pae*QI (*Sac*II), and *Uba*5I (*Sau*3AI) restriction enzymes, respectively. After purification, these enzymes have been characterized on lambda, T7 phage DNA, pUC19, pBR322, PhiX174, M13, SV40 and Adenovirus-2 substrate DNAs.

The newly isolated enzymes have properties of easy purification, simple optimal reaction conditions, and easy growth and maintenance of their source strains. Heat inactivation property of seven enzymes is comparable with their isoschizomers. All enzymes, except *Pae*QI are sensitive to heat. Methylation sensitivities of these enzymes were found to be the same as those for their isoschizomers. The precise sites of cleavage for the seven enzymes have been determined by primed synthesis reactions. All recognize and cleave a known 4-6 nucleotide long stretch of DNA and have the same recognition and cleavage sites as their isoschizomers, except *Bsc*AI from *Bacillus schlegellii*. This enzyme recognizes a non palindromic pentanucleotide sequence 5' GCATC-3' and is the only known isoschizomer of *Sfa*NI. Cleavage site of the enzyme has been determined in comparison with *Sfa*NI, using different adenovirus-2 fragments cloned in M13. *Bsc*AI cleaves four nucleotides downstream the recognition sequence, producing a two nucleotide 5' extension GCATC (4/6); *Sfa*NI cleaves five nucleotides downstream producing four nucleotide 5'extension GCATC (5/9). The difference in DNA sequence between the recognition site and cleavage site does not affect enzyme cleavage specificity. Hence, the newly isolated enzyme has been categorized as type IIS neoschizomer of *Sfa*NI.

The *Ecl*JI *Pvu*I methyltransferase gene from *Enterobacter cloacae* J (recognition sequence : 5'-CGATCG-3') has been cloned into *Escherichia coli* by shotgun method, using *Hin*dIII and plasmid vector pUC19. The selection was based on detection of methylation properties rendering recombinant plasmids carrying the methylase gene nonsusceptible to *Ecl*JI/*Pvu*I endonuclease cleavage. Presence of methylase activity was confirmed by carrying out ^{3}H-methyl incorporation and DNA protection assays.

Also available for viewing as POSTER NO. 14.

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IDENTIFICATION AND CLONING OF MICROCOCCUS LUTEUS EXCISION REPAIR GENES. F. M. Alvi. National Centre of Excellence in Molecular Biology, University of the Punjab, Canal Bank Road, Thokar Niaz Baig, Lahore-53700, Pakistan.

The present study was conducted to identify uvrA, uvrB and uvrC like repair system in *M. luteus* and subsequently clone component genes if present. Hybridization experiments using *E. coli* uVRA, uvrB and uvrC genes as probes, indicate that either such a system is absent in *M. luteus* or component genes of the UV repair complex are structurally different from those in *E. coli*. Western blot analysis using antibodies raised against *E. coli* uvrA, uvrB and uVRC gave negative results suggesting that if cognate proteins are present, they must fold differently to form epitope distinctly different from those of *E. coli* repair antibody proteins. In a separate set of experiments *M. luteus* genomic library was transformed into *E. coli* mutants deficient in uvrA uvrB or uvrC functions. Transformed strains MHI/uvrA and N364/uvrB were able to grow at UV doses as high as 50J/m^2 where the parent strain exhibited 100% loss in survival. However, no recombinant plasmids could be isolated from the transformants and data with Southern blot analysis were also negative. A second approach, employed for the cloning of UV repair genes was based on PCR amplification. Primers were designed and synthesized at CEMB DNA synthesizer, and used for the amplification of *M. luteus* uvrA and urvB genes. These studies are in progress.

Also available for viewing as POSTER NO. 17.

23

USING RFLPs AND RAPDs AS GENETIC MARKERS IN CONTEMPORARY PLANT BREEDING. K. A. Siddiqui and S. F. Mehmood. Atomic Energy Agricultural Research Centre Tandojam, Sindh, Pakistan.

Restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPDs) have opened novel vistas as new class of genetic markers. In recent years their use is increasing with rapid pace in contemporary plant breeding. These genetic markers also provide protection to plant breeder. However, the development of molecular maps and identification of useful markers is labour intensive and requires a skilled molecular geneticist. There are several conditions that must be met to ensure requisite polymorphism in RFLP and promote reproducibility in RAPD amplification. The complexity and sophistication involved in the techniques with respect to the level of polymorphism has promoted a debate on the routine application of RFLP and RAPD in large scale crop improvement. Methodological limitations can be circumvented by following the evolutionary pathways of crop plants. Methods of chemical taxonomy can be combined with new molecular techniques for realizing new opportunities of crop improvement.

24

SOME ASPECTS OF PLANTS MOLECULAR BREEDING AT NIAB. **S. Farooq.**
Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan.

One of the aspects of plant molecular breeding programme of NIAB is to identify gene(s) for abiotic stress tolerance that are residing either in wild species or in cultivated varieties. The objectives of the studies were;

- To identify wild rice species possessing useful genes for rice improvement.
- To identify species specific markers for these wild species.
- To monitor marker assisted flow of genetic material from wild species to cultivated varieties.
- Tagging genes with molecular markers and utilization of markers as probes.

In phase-1 of this programme, we have identified some wild rice species which possesse considerably higher salt tolerance compared to that available in cultivated varieties. These species can be used as donor of salt tolerance gene(s) to cultivated varieties. Since most of these species are morphologically identical though having different ploidy level and genome therefore, we tried to discriminate these species with the help of molecular markers. During the studies, we identified some cultivar and/or species and cultivated rice varieties. In our efforts to use these markers in breeding programme for improvement of salt tolerance of cultivated rice, we hybridized some of these species with cultivated rice and detected the presence of cultivar/species specific markers in the F1 hybrids. some of these markers will co-segregate with salt tolerance in the F2 population. Efforts are being made to clone these fragments in plasmid for multiplication. Such fragments can be used as probes for screening large breeding populations. Some rice cultivars were also transformed with macro-injection of DNA extracted from wild rice species which were difficult to hybridize. In one of such studies where rice cultivar IR-6 was transformed with DNA extracted from kallar grass (*Leptochloa fusca* L.), changes in IR-6 were detected using the peroxidase isozyme system. To test the stability of this transformed plant, genetic studies are being made. Similar studied have also been made using DNA extracted from a wild coastal plant *Porteresia coarctata*. Conditions have also been standardized where any alien DNA fragment(s) irrespective of its sequence can be detected in the hybrid using some suitable primers. Results of some of these studies would be presented.

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CLONING OF MOSQUITOCIDAL TOXIN GENE FROM LOCALLY ISOLATED BACILLUS SPHAERICUS STRAINS INTO E. COLI. **M. Rahman, A. Hossain, E.H. Chowdhary, L.B. Banu and N. Khan.** Department of Biochemistry, University of Dhaka, Dhaka.

A number of *Bacillus sphaericus* (Bs) strains have been isolated from local soil. They have been characterized biochemically and morphologically. Bioassay system has been developed. Most of the isolates have been found to be active against larvae of *Culex quinquafaciatus* which is the major mosquito menace of Dhaka City. Besides it is active against *Aedes*.

Bacillus sphaericus strain SI-2 have been found to be very potent against culex. Attempt have been made to isolate and clone the gene into *E. coli*. A HindIII cut fragment molecular size 3.8 kb has been isolated and cloned into *E. coli* strain DH 5F using plasmid vector PHSKII $^{+}$, A 66 KDa protein was found to be expressed in *E. coli*.

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ISOLATION, CHARACTERIZATION AND CLONING OF CRY GENES FROM LOCAL BACILLUS THURINGIENSIS. **E. Khan**. National Center of Excellence in Molecular Biology, University of the Punjab, Lahore-53700, Pakistan.

To explore the diversity of *Bacillus thuringiensis* and their potential use against local insect pests eight hundred samples of soil, insect cadavers, wild animal dung, decaying leaves and grain dust were collected from different ecological regions of Pakistan to seperate more than 1000 isolates. 400 *B. thuringiensis* found in these samples have been characterized for gene composition by ELISAs, DNA-DNA hybridizations, western blotting and SDS-PAGE. The most frequently occuring crystal protein combinations found are CryIV, CryIA(a)/CryIA(c) and CryIA(a)/CryIA(c)/CryIIA. Seven isolates have been characterized as different/novel on the basis of protein profiles. The insecticidal activity of the isolates is currently being characterized against six local insect pests.

One local isolate D4.10, showing activity against several lepidopteral and dipteral insects was selected for cloning the gene of a 72kd protein. The protein of interest was purified from SDS polyacrylamide gels and analysed for amino terminal sequence. Based on the sequence and codon usage in other Bt toxins a 33mer degenerate oligonucleotide probe was synthesized and used to isolate the 72kd protein gene from a large plasmid. The cloned gene was characterized by restriction mapping and partial sequencing to have a high homology with the CryIVD gene. The CryIVD gene under its own promoter did not produce any detectable levels of the 72Kd protein in *E. coli*. Therefore, the gene has been subcloned in pMK4 shuttle vector and pDH32 integration vector for expression in *B. subtilis*.

Also available for viewing as POSTER NO. 9.

27

CLONING OF BT CRY GENE WITH HIGH ENTOMOCIDAL ACTIVITY AGAINST COTTON BOLLWORMS. **R. Makhdoom**. National Centre of Excellence in Molecular Biology, University of the Punjab, Thokar Niaz Baig, Canal Bank Road, Lahore-53700, Pakistan.

Cotton spotted bollworm,(*Earias vitella*) and American bollworm, (*Heliothis armigera*), two devastating pests of cotton were successfully reared on artificial diet. The artificial diet standardized for the insect larvae was completely adequate to take the insects through a number of generations. Biotoxicity assays with more than 50 local Bt isolates were done against bollworms up to LC50 level to select for the isolates harbouring high entomocidal activity. One isolate JR6.3 was found to be highly toxic against spotted and American bollworm (LC50 values of 11ng/mg and 7ng/mg of diet respectively) in comparison with the commercial HD1-*kurstaki* strain (LC50 values of 25ng/mg and 28ng/mg respectively) and with the HD73 strain (LC50 values of 23ng/mg and 30ng/mg of diet respectively).

In order to clone the *cryIA(c)* gene from the isolate JR6.3 a HindIII plasmid library was made in bluescript vector and positive clones selected by screening the transformants with a *cryIA(b)* probe. The positive clones were characterized by restriction mapping, PCR amplification, and partial sequencing. The expression studies of *cryIA(c)* gene in *Bacillus subtilis* are underway.

Also available for viewing as POSTER NO. 10.

28

CLONING OF BT DELTA ENDOTOXIN GENE CONTAINING LARVICIDAL ACTIVITY AGAINST COTTON PINK BOLLWORM. S. Karim. National Centre of Excellence in Molecular Biology, University of the Punjab, Canal Bank Road, Lahore-53700, Pakistan.

A large number of samples of organically rich soils, bird droppings, animal dung and seed dust, collected from different ecological regions of Pakistan, were analyzed by sodium acetate selection method to separate *Bacillus thuringiensis* isolates. Local Bt isolates were characterized for pesticidal properties against a variety of larvae. Potentially larvicidal isolates were further studied for DNA homology with known crystal protein genes. Based on these studies, PR17.4 was selected as a potentially efficaceous source for the isolation and cloning of CryIA gene into *E. coli*. A *Hin*dIII plasmid library of PR17.4 DNA was constructed in bluescript vector. More than three thousand transformants were screened to give five positive clones. PCR amplification was used to amplify a 0.5 Kb fragment of one positive clone PR19.12 by using internal primers of conserved regions of CryIA gene. Restriction mapping of the cloned 4.5 Kb HindIII fragment was done to characterize the presence of coding region of the CryIA(a) gene. Western blot analysis was done to study the level of expression of cry protein. No detectable levels of cryI protein was expressed in *E. coli*. Expression of cryIA(a) toxin in *E. coli* under tac promoter is underway.

Also available for viewing as POSTER NO. 11.

29

IDENTIFICATION OF BASMATI RICE CULTIVARS AND THEIR RADIATION INDUCED MUTANTS WITH RANDOM AMPLIFIED POLYMORPHIC DNA (RAPDs). **N. Iqbal, M. Arif, A. A. Zaidi and S. Farooq**. Nuclear Institute for Agriculture and Biology (NIAB), Jhang Road, Faisalabad, Pakistan.

Random Amplified Polymorphic DNA markers were generated in a Perkin Elmer's DNA thermal cycler using genomic DNAs extracted from three Basmati rice cultivars, their early flowering and dwarf mutants with 40 arbitrary 10-mer oligonucleotide primers. The objectives of the study were to differentiate i) 3 Basmati varieties from each other ii) Basmati varieties from mutants and iii) different mutants from each other. Of the 40 primers, some primers have been identified which unambiguously differentiated different varieties from mutants and different mutants by at least one cultivar and/or mutant specific RAPD marker. These markers are now being used to identify the varieties and mutants (DNA FINGERPRINTING). Efforts are now being made to see the potential of these markers for gene tagging experiments using F_2 population of a rice variety and its polymorphic mutant.

Also available for viewing as POSTER NO. 3.

30

MOLECULAR PROPERTIES AND PHYLOGENETIC ANALYSIS OF COTTON LEAF CURL VIRUS, A NEW WHITEFLY-TRANSMITTED GEMINIVIRUS FROM PAKISTAN. S. Mansoor, P. Markham[1], J. Stanley[1], Y. Zafar and K. A. Malik. National Institute for Biotechnology and Genetic Engineering (NIBGE), P.O. Box 577, Faisalabad, Pakistan. [1]Department of Virus Research, John Innes Centre, Norwich, UK.

For the last three years cotton leaf curl disease has devastated cotton crop in Pakistan and has resulted in about 40% decrease in cotton yield. We have shown that cotton leaf curl virus (CLCuV) is a whitefly transmitted geminivirus. In the present communication molecular and some biological properties of CLCuV will be discussed.

Overlapping primers were designed on the basis of available sequence of CLCuV. The complete genome of CLCuV amplified by these primers was cloned in appropriate vector. A combination of primer extension and subcloning was used for the determination of complete sequence of CLCuV. The data was assembled and compared with known dicot-infecting geminiviruses. The sequence was more similar to whitefy-transmitted geminiviruses as compared to leafhopper-transmitted geminiviruses. Among whitefly transmitted geminivirus CLCuV was more related to old world geminiviruses, especially those found in Indian subcontinent. Phylogenetic analysis of CLCuV for each open reading frame will also be presented.

A partial dimer of full length clone of CLCuV was cloned in T-DNA borders of an *Agrobacterium* vector. The resulting dimer was used for virus replication studies both in suspension cell culture and leaf discs. The same clone was also used for agroinoculation studies on certain indicator hosts and cotton plants.

The evaluation of diversity of CLCuV in field conditions is essential for effective management and development of vius-resistant varieties, either through conventional methods or genetic engineering. A strategy for the determination of field diversity has been developed which include Southern hybridization under stringent conditions, RFLP analysis of replicative form of virus or PCR amplified DNA and limited sequence analysis. For this purpose a number of virus isolates from different parts of cotton growing areas of Pakistan affected by CLCuV has been collected and CLCuV has been detected by Southern hybridization. Correlation of biological and molecular properties of CLCuV with the management of cotton virus under field condition and development of virus-resistant varieties through genetic engineering will be discussed.

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ASSESSMENT OF GENETIC SIMILARITIES OF VARIOUS RHODODENDRONS BY RAPD PROFILES. M. J. Iqbal and A. L. Rayburn[1]. National Institute for Biotechnology & Genetic Engineering (NIBGE), P.O. Box 577, Faisalabad, Pakistan. [1]Department of Agronomy, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

A large number of new *Rhododendron* varieties and hybrids are produced each year in the U.S.A. It is important to study their genetic relationships which can be helpful for their further improvement. Intravarietal genetic homogeneity was established in three *Rhododendron* species based on their amplification profiles of random amplified DNA (RAPD) markers. Amplification patterns of vegetatively propagated *R. atlanticum* (10 plants), *R. arborescens* (9 Plants) and *R. yedonese* var. pouhkanense (10 plants) were consistent with 10 primers studied. Only one plant of *R. yedoense* var. *poukhanense* showed a difference of one band with one primer. As compared to vegetatively propagated rhododendrons, when F2 plants of two hybrids were analyzed, extensive polymorphisms were revealed. RAPD profiles produced with 10 primers were then used to study the genetic similarities of thirteen Rhododendron species, varieties and hybrids. Amplification patterns were then compared with each other on the basis of the size of the amplified fragments. The genetic relationships revealed by cluster analysis were similar to that of their known pedigree.

32

INCORPORATION OF PEST RESISTANCE IN BASMATI RICE. **F. Khanum, T. Husnain and E. Khan**. Centre of Excellence in Molecular Biology, University of the Punjab, Canal Bank Road, Thokar Niaz Baig, Lahore-53700, Pakistan.

Basmati rice (*Oryza sativa* sp. indica) is attacked by two major insects, the yellow stem borer (*Tryporyza incertulas*) and the rice leaf folder (*Cnaphalocrocis medinalis*). In Pakistan, the estimated damage by these insects is 20-25% in yield per annum. In order to insert the insect resistant gene from *Bacillus thuringiensis* into Basmati rice, twelve varieties of *Oryza sativa* were investigated for their ability to regenerate into whole plants either from meristem tissues or from embryogenic calli. These plants were transferred into the soil grew to maturity, and bore seeds that were 100% viable. The method of meristem culture was used for the transformation of rice with foreign genes using particle acceleration.

Conditions have been established for transient as well as stable expression of genes in leaf bases and mature embryos. Beta-glucuronidase gene was introduced into leaf bases for transient expression while selectable marker gene (*hph*) which confer hygromycin resistance in transgenic tissues was used for stable transformation in mature embryos. Green fast growing plants were obtained on selection of hygromycin at concentration of 50ug/ml.

Preliminary analysis of genomic DNA of transgenic plants was carried out by dot-blot hybridization of Bt and hph gene which showed integration of foreign genes. Insect feeding assay with 3rd instar larvae of leaf folder showed the presence of insect resistance. Southern hybridization data confirmed the stable integration of CryIA(c) gene into the genome of these plants.

Also available for viewing as POSTER NO. 1.

33

INSECT RESISTANCE CHICKPEA GENERATED BY INTRODUCTION OF A MODIFIED DELTA-ENDOTOXIN GENE OF BACILLUS THURINGIENSIS. **T. Malik**. National Centre of Excellence in Molecular Biology, Thokar Niaz Biag Canal Bank Road Lahore-53700, Pakistan.

The biolistic particle delivery system has been used as a wounding mechanism to enhance the *Agrobacterium* mediated transformation of gram legume *Cicer arietinum* L. (chickpea). Mature embryos were bombarded prior to or after the application of *Agrobacterium* tumefaciens strains containing the GUS, NPTII and CryIA(b) genes within the T-DNA. Transformants were selected on high concentration of kanamycin 100mg/l. This treatment resulted in the production of significantly high number of plants that were resistant to kanamycin when compared with the number of plants generated through standard biolistic transformation protocol using pBI121 vector. Further, the proportion of production of chimeric plantlets was reduced to negligible level as evidenced by histochemical staining for GUS expression. Eight week old kanamycin resistant shoots were screened by dot blots and approximately 10-12% of the shoots were found to be stable transformants as confirmed by Southern blot analysis.

To study the expression of foreign DNA of transformed plants to protect against insect damage was studied through bioassays. Second instar *Heliothis armigera* larvae were allowed to feed on eight week old shoots. Mortality was monitored after 5-6 days. Larvae fed on transformed plants exhibited 75-100% mortality when compared with that on untransformed plants. Expression of CryIA(b) in the transformed chickpea was confirmed by Western blotting. These results confirm the entry and expression of crystal protein Bt gene in chickpea plants.

Also available for viewing as POSTER NO. 4.

34

A MODERATELY ABUNDANT DNA SEQUENCE FROM MAIZE INCREASES TRANSIENT GENE EXPRESSION. A. Bashir and A. L. Rayburn[1]. National Institute for Biotechnology and Genetic Engineering (NIBGE), P.O. Box 577, Faisalabad, Pakistan. [1]Department of Agronomy, University of Illinois at Urbana-Champaign, Urbana, Il 61801, USA.

A large portion of the maize genome is composed of repetitive DNA sequences. The most abundant of which are reported to be knob and rDNA sequences. Six maize genomic clones were identified to contain moderately abundant DNA sequences other than knob and ribosomal DNA sequence. The relative abundance of these genomic inserts was determined in six maize lines capable of plant regeneration in tissue culture. One of the clones was found to be equally abundant in all the six maize lines. The genomic insert in the clone was sequenced. This 662 bp sequence was used to flank the hygromycin phosphotransferase (hpt) and *Uid*A genes in plasmid pBluescript in a direct repeat order. The resulting construct was used in both circular and linear forms to transform type I embryogenic calli of the maize line H99 through particle bombardment. The statistical analysis indicated a significant increase in transient expression efficiency. The results on the transient expression of circular and linear DNA molecules will be discussed.

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RICE TRANSFORMATION BY BIOLISTIC METHOD. **C. Z. Mukhtar, Y. Zafar, K. A. Malik, A. Kokli[1], C. Manguito[1], B. Ghareyazi[1] and J. Benntt[1].** National Institute for Biotechnology and Genetic Engineering (NIBGE) P.O. Box 577, Jhang Road, Faisalabad, Pakistan. [1]Plant Molecular Biology Lab. Division of Plant Breeding, Genetics and Biochemistry, International Rice Research Institute (IRRI), P.O. Box 933, 1099 Manila, The Philippines.

Basmati rice (*Oryza sativa* L.) varieties B-370, B-Kashmir and B-Pak were used for this study. Immature embryos and primary calli from mature seeds were used as the potential targets for biolistic gun transformation. Construction of recombinant plasmids for use in rice transformation will be discussed. Different parameters controlling bombardment efficiency were also studied. Molecular analysis of the putative transgenic plants was done by PCR. Further confirmation is being made by Southern hybridization and progeny analysis. The development of this technology would aid in opening the era of transgenic crops in Pakistan.

Also available for viewing as POSTER NO. 2.

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40 TRANSFORMATION OF COTTON (G. HIRSUTUM L.) BY AGROBACTERIUM AND MICROPROJECTILE BOMBARDMENT DNA DELIVERY SYSTEMS. **N. A. Saeed, S. Asad, Y. Zafar and K. A. Malik.** National Institute for Biotechnology and Genetic Engineering, P.O.Box 577, Jhang Road, Faisalabad, Pakistan.

Attempts to obtain genetic transformation of cotton have been performed using *Agrobacterium* and biolistic gun methods. Hypocotyl sections of Siokra 1-3 and Coker-312 cotton varieties were inoculated with two different strains of *Agrobacterium*. Transformed kanamycin resistant calli were produced at high frequencies. These calli induced to form somatic embryos which were then germinated and formed plants. Positive GUS expression was observed at callus and at the whole plant level.

The development of particle bombardment as a mean to deliver DNA into plant cells and tissues provide an alternative procedure for transformation. Analysis of physical parameters inherent in this technology were undertaken in meristem tips and mature embryos of three cotton varieties viz. NIAB-78, MNH-93 and S-12 Transformation of shoot meristem tip and mature embryos occurred. Integration of the exogenic DNA (pTG0072GUS) in the cotton genome will be determined in putative cotton transformants. These efforts for producing transgenic plants facilitate transfer of genes of economic importance in local cultivars of cotton.

Also avaiable for viewing as POSTER NO. 5.

37

METABOLIC ENGINEERING SOLUTIONS TO THE PROBLEMS OF LARGE SCALE ANIMAL CELL PROCESSING FOR THE PRODUCTION OF BIOPHARMACEUTICALS. M. Al-Rubeai. BBSRC Centre For Biochemical Engineering, School of Chemical Engineering, The University of Birmingham, Birmingham B15 2TT, UK.

The advancement of the technology for large scale cultivation of animal cells has relied on the implementation of strategies and techniques which can be grouped into four categories: maximisation of viable cell concentration, improvement of medium formulation, implementation of high-performance reactor configurations and maximisation of specific production rate. However, the development of optimal processing schemes has always been hindered by the lack of necessary information on the processes of cell proliferation and death and on the regulation of product synthesis and secretion. We have evaluated the changes in monoclonal antibody (MAb) productivity in hybridoma culture as influenced b the cell cycle time and cell death in batch, continuous and perfusion cultures. Synthesis and secretion of MAb were shown to be at maximum rates in the G1/early S phases and that the secretory process is inefficient, leading to accumulation of MAb which is only released passively from dying and necrotic cells. Death process, however, was mainly by apoptosis (programmed cell death) which follows rapidly after the cessation of growth. The death of cells undergoing apoptosis is characterised by chromatin cleavage at the internucleosomal sites which results in extensive fragmentation of DNA. Having already identified the cell lines in which apoptosis plays an important role during the fermentation process and that apoptosis responses can result from nutrient limitation, cytotoxic product accumulation and hydrodynamic effects arising from inappropriate agitation or aeration conditions. It has now been shown that the over-expression of the bcl-2 gene can protect the cells from death induced in suspension culture by the hydrodynamic environment. It was also possible to maintain cell survival in serum free medium by over-expression of bcl-2. The induction of apoptosis following growth arrest is a major barrier to the development of culture strategies that optimise specific productivity by reducing the growth rate. Our results suggest that suppression of apoptosis by enhanced bcl-2 expression under these conditions allows the maintenance of cell culture in a growth-arrested state for much longer than would otherwise be possible.

38

NEUROTRANSMITTERS-MEDIATING ENZYMES IN HEALTH AND DISEASES OF MAMMALS INCLUDING THE HUMAN SUBJECTS. M. K. Rehman[1], M. Salimulla[1], M. A. Islam[1], N. G. Banik[2] and T. Nagatsu[3]. [1]Department of Biochemistry, University of Dhaka, Dhaka-1000, Bangladesh, [2]BIRDEM, Kazi Nazrul Islam Avenue, Dhaka-1000, Bangladesh and [3]Tokyo Institute of Technology, Tokyo, Japan.

The activity of aromatic L-amino acid decarboxylase (AADC) with L-DOPA and L-5-hydroxytryptopan (L-5-HTP) as substrates was measured in various regions of human brains from controls and patients with Parkinson's disease, striato-nigral degeneration, Shy-Drager syndrome, and perioral dyskinesia. This is the first time that the distribution of L-5-HTP decarboxylase activity was measured in human brain regions. The catecholaminergic regions of control brains such as the amygdala, hypothalamus, caudate nucleus, and putamen preferentially decarboxylated L-DOPA, whereas a serotonergic region such as the raphe nucleus had relatively high activity for L-5-HTP. Both DOPA decarboxylase and 5-HTP decarboxylase activities and therefore, the ratio between the two kinds of activity were variable in the brains from controls and patients. We also studied the effect of Lahyrus Sativus seeds (LSS) on AADC in the central and peripheral tissues and serum of LESS-treated and LSS plus vitamin C-treated guinea pigs. The feeding of LSS for 35 days decreased the AADC activity significantly in the brain and peripheral tissues, but the activity was recovered to moral level in the most tissues when vitamin C was added with the LSS. We also undertook the studies of dopamine-beta-hydroxylase (DBH) activity in the central and peripheral tissues and serum of normal, malnourished and diabetic rats. In malnutrition, DBH activity increased significately in serum and peripheral tissues, such as liver, small intestine, heart, kidney,; but the activity did not change significantly in the brain tissues such as cerebral cortex, brain stem, hypothalamus, caudate nucleus, colliculi and in diabetic rats, similar results were obtained in the peripheral tissues for DBH. But the effects on DBH activity was variable in the brain tissues. The DBH activity in the serum of malnourished-and diabetic-patients was variable.

39

MOLECULAR STUDIES ON THE DISTRIBUTION OF BETA-THALASSEMIA IN QUESHM ISLAND. **N. Moazami and F. Bermek**. Biotechnology Centre, I.R.O.S.T. Tehran, Iran and Marmara Research Centre, Turkey.

The Beta-Thalassemia is one of the major genetic disorders in Iran. About 18,000 Beta-Thalassemic patients are living in different parts of the country. By application of recombinant DNA technology, the polymerase chain reaction and dot blot hybridization techniques, we have investigated the molecular basis of Beta-Thalassemia from Queshm Island.

Queshm is one of the biggest Islands in Persian Gulf, with a total population around 70,000. Joint family system is still running among all the families and marriages are among families too.

6 oligonucleotide probes for the mutations of IVS, 1/nt 110, IVS, 1/nt6, IVS,1/nt, I, Nonsense codon 39, Frameshift codon 8 and IVS.2/nt.1 were selected with respect to their relative frequency in the region.

The results of hybridization with oligonucleotide probe correspond that the most frequent was the nonsense codon 39 mutation (95%) and the remainder was frameshift codon 8.

The nonsense codon 39 mutation (C/T) which accounts for about 95% of the Beta-Thalassemia cases in sardina is regarded as a rather Western Mediterranean prototype. The very high frequency of the nonsense codon 39 mutation on Queshm Island reveals an interesting parallel to Sardina.

40

SCREENING FOR THE MOLECULAR DEFECTS CAUSING BETA THALASSEMIA IN PAKISTAN. S. N. Khan. National Centre of Excellence in Molecular Biology, University of the Punjab, Canal Bank Road, Thokar Niaz Baig, Lahore-53700, Pakistan.

Beta thalassemia is the commonest inherited hemoglobin disorder in Pakistan with an overall carrier rate of 5.4%. It is an autosomal recessive disorder associated with a reduced or absence of synthesis of globin chains of the hemoglobin molecule, causing severe anemia in homozygotes (more commonly known as thalassemia major patients). Therapy is supportive and frequent blood transfusion is necessary to prevent death from severe anemia. Genetic counselling and prenatal diagnosis (diagnosis of the fetus by chorionic villi samples as early as 8-10 weeks of pregnancy) can play an important role in reducing the incidence of this disease.

There are over 120 molecular defects causing beta thalassemia all over the world. However each population at risk usually has its own specific set of mutations that makes the prenatal diagnosis fairly easy. The information so far available for this region is for Asian Indians. We have been collecting blood samples from clinically diagnosed beta thalassemia patients from all four provinces for screening the molecular defects causing beta thalassemia in Pakistan. Blood samples from 300 unrelated patients (comprising of 600 chromosomes) have been collected. Allele Specific Oligonucleotide Probes (ASO) were used after amplification of beta globin gene by Polymerase Chain Reaction (PCR) to detect different mutations either by immunological or by chemiluminescent detection. Of the 156 chromosomes analyzed so far, the mutations identified are codon 8/9(+G) in 42, IVSI-nt. 5 (G-->C) in 38, ISVI-nt.1 (G-->T) in 14 and Frameshift 16(-C) in 2. Screening for other mutations is in progress.

Also available for viewing as POSTER NO. 20.

41

DEGRADATIVE ENZYMES, CYTOKINES AND DISEASE PATHOGENESIS. **S. U. Kazmi, F. Anwar, R.C. Rees[1] and C.W. Potter[1].** IIDRL, Department of Microbiology, University of Karachi, Karachi 75270, Pakistan. [1]University of Sheffield Medical School, UK.

Loss of connective tissue integrity occurs in many disease processes including rheumatoid arthritis, cancer osteoarthritis, tumor cell invasion, bacterial and parasitic infestation. Although there is a high incidence of such disease in the developed as well as developing world, there is no treatment that prevents the extensive tissue damage which occurs in such patients. Several lines of evidence suggest that tissue damage is due to uncontrolled expression of connective tissue degradative enzymes especially metalloproteinases. Inhibition of such enzymes in the presence of different cytokines, therefore has become the target for therapeutic intervention in various diseases. A number of such enzymes and their inhibitors have been described but due to difficulties in isolation in sufficient quantity as pure separate enzymes, very little knowledge has accumulated about their detailed molecular biology and biochemistry. Matrix metalloproteinases are capable of degrading both collagenous and non collagenous components of the extracellular matrix. These enzymes have broad proteinase activity, indicating that they may have a role in tissue turnover process. The aim of the present study was, first to investigate degradative enzyme expression by human and animal cell/cell lines and their possible modulation by cytokines and substances of bacterial origin. The enzyme activity was detected by gelatin and casein embedded PAGE and Zymography. Antitumor activity of resting and cytokine activated human lymphocytes was also determined by an *in vitro* assay. Our results inferred that human lymphocytes, monocytes and different cell lines do express various degradative enzymes but differ in their quality and amount of enzyme expression. We also report modulation of enzyme expression in presence of Interleukin-1 and Interleukin-2 in monocyte and lymphocyte cultures respectively. Our results also indicated that IL-2 modulates the enzyme expression by lymphocytes but not by other cell lines. Cytotoxic expression by lymphocytes but not by other cell lines. Cytotoxic effects of resting and IL-2 activated Human Lymphocytes were detectd against 3 different tumor cell lines i.e Human Melanoma Cells, Human Colon Adenocarcinoma and Human Myeloid Leukemia cell line. IL-2 activated lymphocytes cause lysis of target cell very efficiently as compared to resting cells, however prior treatment wit Alpha-Interferon protected the cells from lytic effects.

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PURIFICATION AND CHARACTERIZATION OF NEOMYCIN PHOSPHOTRANSFERASE-II AND ITS IMMUNOCHEMICAL DETECTION IN TRANSFORMED CHICKPEA. Z. Q. Samra and K. J. Cross. National Centre of Excellence in Molecular Biology, University of the Punjab, Thokar Niaz Baig, Canal Bank Road, Lahore 53700, Pakistan.

Neomycin phosphotransferase II (NPT-II), a protein of 25 KDa, was purified to homogeneity from *neo*-gene cloned in E. coli (JM101). Expressed chimeric protein was efficiently secreted into the periplasm and maximally released by osmotic shock. High yield of purified enzyme (96-98 %) with specific activity 6.5 - 7 Units/mg was recovered by ammonium sulfate precipitation, separation by Gel permeation, followed by Ion-Exchange and Affinity chromatography on a new affinity resin (neomycin-sepharose 4-B). Enzyme activity was monitored spectrophotometrically using kinase enzyme assay with the subsequent reduction of $NADH_2$ at 340 nm. The protocol provides protein with a higher specific activity than that previously obtained(1). Specific antibodies against NPT-II were generated and characterized by immunochemical tests. These antibodies were used to detect the NPT-II expression in *neo*-gene transformed chickpea callus homogenate and confirm the presence of 25 KDa protein band in immunoblot analysis. Practical uses of the antibodies prepared against NPT-II will be presented.

Also available for viewing as POSTER NO. 21.

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DETECTION OF MYCOBACTERIUM TUBERCULOSIS **IN FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE BY POLYMERASE CHAIN REACTION.** **R. Tabassum, S. Ahmed[1], J. A. Qureshi and K. A. Malik.** Basic Biology Division National Institute for Biotechnology & Genetic Engineering (NIBGE), Faisalabad, Pakistan. [1]Department of Pathology, Post Graduate Medical Institute, Lahore, Pakistan.

Infection with *Mycobacterium tuberculosis* is a major cause of death world wide. For tissue diagnosis of tuberculosis variable site biopsies are processed for histological assessments and microscopy. They, however, reveal only the lesions produced by the *Mycobacterium tuberculosis*, microscopically graded as "granulomas". No *M. tuberculosis* is visualized histologically and hence is reported as the lesion picture consistent with TB lesion-Tuberculous.

Direct visualization can be performed by Ziehl-Neelson staining and fluorochrome stains but this method is unsatisfactory. The amplification of specific DNA sequences by polymerase chain reaction (PCR) is a novel tool for the detection of different infectious organisms and has already been applied to detect mycobacterial DNA sequences in sputum, pleural fluid and cerebrospinal fluid.

The authors therefore compared conventional staining techniques, histological findings and polymerase chain reaction for the detection of mycobacterial DNA sequences (IS 6110) in formalin fixed, paraffin embedded tissues. DNA was extracted from 5 micron unstained paraffin sections by proteinase K digestion and amplified by PCR with primers for the IS6110. Mycobacterial DNA was either visualized directly on a agarose gel or detected by dot blot hybridization. Our results show that the polymerase chain reaction can detect low numbers of acid fast organisms in paraffin sections and confirm and pre-sumptively speciate mycobacterial infection when cultures are negative or not obtained.

Also available for viewing as POSTER NO. 22.

44

MOLECULAR BIOLOGY OF RETINITIS PIGMENTOSA. **J. R. Singh, V. Kaur, A. P. Singh, and D. Singh**. Centre For Genetic Disorders, Guru Nanak Dev University Amritsar 14005, India. Dr Daljit Singh Eye Hospital 57 Joshi Colony. Amritsar, India.

Retinitis pigmentosa (RP) is the major cause of inherited form of blindness. It occurs with a frequency of approximately 1:4000 and affects all populations of the world and its cure is still elusive. RP primarily affects photoreceptors and pigment epithelial function and is characterized by nyctalopia (night blindness), extinguished ERG response, constriction of visual fields, ultimately leading to the death of rod photoreceptor cells. Autosomal dominant, autosomal recessive and X-linked recessive modes of inheritance have been reported for it. The complexity of retinitis pigmentosa is increased by the observation that it 7is not a single disease entity, but represents a group of inherited disorders of the eye. All of these disorders primarily involve the retina, with nyctalopia as their major clinical symptom, and they all ultimately lead to permanent blindness. Candidate gene approach is the only way to identify and characterise the genes underlying retinitis pigmentosa. During the past four years we have investigated over 120 families exhibiting different inheritance patterns of RP. Following the candidate gene approach, molecular genetic studies are being undertaken for the identification of genes responsible for RP. The rational behind selecting the various genes, the utilisation of these in molecular diagnostics of this disease, and the results obtained would be discussed.

45

AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA LOCUS MAPS ON CHROMOSOME 1Q IN A LARGE CONSANGUINEOUS FAMILY FROM PAKISTAN. F. Younus, J. L. Weber[1], S. Q. Mehdi and A. Gal[2]. Biomedical and Genetic Engineering Division, Dr. A.Q. Khan Research Laboratories, Islamabad-44000, Pakistan. [1]Marshfield Medical Research Foundation, Marshfield, USA. [2]Lubeck University, Germany.

Retinitis pigmentosa (RP) is a group of genetically inherited diseases which involve degeneration of the retina. Patients having retinal degenerations typically have night blindness and loss of mid peripheral visual field. In advanced stages, examination of the fundus reveals retinal vessel attenuation, intraretinal pigment in the peripheral fundus and waxy pallor of the optic disc. Finally, there is a widespread loss of photoreceptors leading to blindness.

It is well known that RP can be inherited in the autosomal recessive, autosomal dominant and X-linked forms. The first genetic linkage study showed the segregation of a polymorphic DNA marker DXS57 in X-linked forms of RP. The gene for autosomal dominant RP was found to be linked to an anonymous polymorphic marker D3S47 on chromosome 3q. Mutations in the genes encoding rhodopsin and peripherin have also been found to be responsible for some forms of RP.

We have studied a large Pakistani family, with several consanguineous marriages, in which autosomal recessive RP was segregating. The entire pedigree consists of 4 major branches. However, there was clear difference between the phenotype of three branches of the family, which had typical features of preserved para-arteriolar retinal pigment epithelium (PPRPE) and the fourth branch which presented the classical form of RP.

A large scale search was performed for linkage using microsatellite polymorphisms distributed all over the human genome. DNA samples were PCR amplified using the oligonucleotide primers in experimental conditions described by Gyapay et al. (1994). These studies revealed close linkage between the disease locus and six loci on chromomome 1q (DIS158, F13B, D1S422, D1S 412, D1S413, and D1S53) with maximum lod scores ranging from 0.988-4.657 at $\theta = 0.065$-0.235. However, the analysis of individual nuclear families showed very close linkage without recombination in three branches and several recombinants and negative lod scores throughout in the fouth branch. These results strongly suggest that mutations of two different genes are responsible for the disease in the 'linked' and 'unlinked' branches. Parallel to the linkage heterogeneity, clear phenotypic differences have been observed among the 'linked' and 'unlinked' parts. Our findings demonstrate that in case of recessive disorders the possibility of non-allelic genetic heterogeneity should always be considered, even within the same kindred and in genetic isolates if a largely extended pedigree is analyzed.

Gyapay G., et al. (1994) Nature Genetics. 7: 246-339.

46

POLYMORPHISM OF THE T-CELL RECEPTOR (TCR) GENE LOCUS: NO EVIDENCE FOR LINKAGE WITH SUSCEPTIBILITY TO MYCOBACTERIAL DISEASE IN MAN. S. Khaliq, A. Hameed and S. Q. Mehdi. Biomedical and Genetic Engineering Division, Dr. A. Q. Khan Research Laboratories, Islamabad-44000, Pakistan.

Restriction fragment length polymorphisms (RFLPs) of various segments of the T-cell receptor (TCR) -, -, and - and -chains have recently been examined at our laboratory and several others around the world. Although inconclusive, they have suggested that the disease susceptibility genes (or locus, DSL) may be located in the TCR gene complex (Kumar et al., 1989).

Tuberculosis continues to be a major communicable disease, specially in developing countries. The body's own immune surveillance system provides the natural protection against mycobacteria. The importance of the role played by TH1 cells in response to *Mycobacterium tuberculosis*/bovis is well established. The possibility that susceptibility to *M. tuberculosis* infection could be due to polymorphisms of the TCR genes was therefore examined in multicase TB families.

To examine the possibility of allelic segregation in mycobacterial diseases, we examined the polymorphisms in the human T-cell antigen receptors in normal Pakistani populations. A number of gene probes for all four TCR gene segments show allelic polymorphisms with specific restriction endonucleases.

DNA samples from thirteen multicase TB families were digested with five different restriction enzymes and Southern blots were hybridized with probes for the TCR -and -chain genes. Our results show that polymorphic bands are clearly observed but that there is no particular difference in the bands observed with the TB patients and their unaffected contacts. A similar unrelated pattern was observed when DNA samples from families TB1, TB7 and TB16 were digested with *Msp*I and probed with JCB. With several multicase TB families, disease association could not be demonstrated with other combinations of enzymes and probes.

The field of TCR allele variability and disease association has not lived up to earlier expectations due to conflicting findings. The possibility of the existence of disease susceptibility genes within the TCR gene complex is not conclusively supported by the experimental data that is currently available.

The fine specificity of the TCR is essential for all T-cell dependent reactions against antigens. Studies showing strongly restricted TCR gene involvement in pathogenic T-cell clones are not necessarily arguments for a role of inherited TCR gene polymorphism and disease susceptibility (Hillert and Olerup, 1992).

The studies reported here show a lack of linkage between TCR and chain genes and tuberculous disease.

Hillert J. and Olerup O. (1992). Germ-line polymorphism of TCR genes and disease susceptibility-fact or hypothesis? Immunology Today. 13: 47-49.

Kumar V., Kono D.H., Urban J.L. and Hood L. (1989). The T-cell receptor repertoire and autoimmune diseases. Ann. Rev. Immunol. 7: 657-682.

47

EXPRESSION OF GLYCOGEN PHOSPHORYLASE GENES DURING DEVELOPMENT IN NORMAL AND DIABETIC RAT LIVER AND LUNG. A. Ara. National Centre of Excellence in Molecular Biology, Thokar Niaz Baig, Canal Bank Road, Lahore-53700, Pakistan.

Glycogen phosphorylase (GP) plays a vital role in maintaining glucose homeostasis in mammalian tissues. Three isozymic forms of glycogen phosphorylase are specifically found in liver, muscle and brain. These isozymes are similar in structure and enzymatic activity but are mapped on different chromosomes. The presence of three similar isozymes is likely related to tissue specific expression of Glycogen phosphorylase. The cDNAs encoding liver, muscle and brain phosphorylase have been cloned by various laboratories. The cDNA encoding liver phosphorylase have been cloned in our laboratory. We have used two different hepatic phosphorylase cDNA probes to study the expression of GP in normal and diabetic liver and lung during development. Pulmonary Glycogen contents were observed maximum on day 20 of gestation. Pulmonary DNA and protein concentration did not change significantly in normal and diabetic animal. Developmental increase in phosphorylase enzyme activity was also observed higher on day 22 of gestation. Northern blot analysis indicated maximal expression of liver GP on day 19th of gestation and no expression was observed in neonate. However, lung GP did not change during development. Maximal rate of transcription was also observed on day 19th of gestation which decreased in neonate rats.

To understand tissue specific expression of glycogen phosphorylase gene, we have used GP cDNA as a probe to isolate the corresponding genomic clones. Sprague Dawley rat genomic DNA library was screened under stringent condition which yielded six positive clones. Restriction enzyme analysis and southern blot analysis identified a 7.0 kb *Eco*R1 fragment from one of the positive clone that showed strong hybridization with 5'region upstream of liver glycogen phosphorylase gene. Further analysis of 7.0 kb *Eco*R1 fragment has yielded a 1.8 kb *Pst*1 fragment which showed strong hybridization with the same probe. This fragment has been subcloned in to pBSSK$^+$ plasmid in both orientations to generate unidirectional deletions. For the identification of promoter region, the chloramphenicol acetyl transferase (CAT) assay was performed on deleted mutants. Maximum CAT activity was observed in three clones which further confirm the presence of promoter region in it.

Also available for viewing as POSTER NO. 29.

48

PRELIMINARY STUDIES TOWARDS DEVELOPMENT OF BREAST CANCER DIAGNOSTIC KIT TO DETECT HUMAN ESTROGEN RECEPTOR (hER). M. Amjad. National Centre of Excelience in Molecular Biology, University of the Punjab, Thokar Niaz Baig, Canal Bank Road, Lahore-53700, Pakistan.

Qualitative analysis of **hER** purification was undertaken to adapt technology for breast cancer diagnosis in Pakistan. This is a step towards development of a diagnostic kit similar to the Abbot **hER**-enzyme immunoassay kit.

hER, a 66 Kd protein, was expressed in **Saccharomyces cerevisiae**. To perform affinity chromatography a conjugate containing ligand of **hER**, Diethylstilbesterol (DES) or 17B-Estradiol(E2) was coupled to CnBr activated sepharose. With 2M (NH4)2SO4 as eluent **hER** appeared as a faint band in 60-66 Kd size range. Yield was estimated to be about 0.3 ug/ul.
To enrich **hER** content of the crude yeast extract optimum % saturation for an ammonium sulfate cut was determined to be 30% . Use of isoelectric focusing (IEF) was also employed purify the protein. About 32 mg of crude yeast extract carrying about 0.064 mg of **hER** was focused in Biorad's Rotofor using broad pH range (3-10) ampholytes. Following immuno dot blot eight fractions, in the pH range 6.0-8.3, were pooled. Refocusing of these fractions narrowed down the **hER** positive fractions to 6.0-6.8 pH range, thus further resolving the desired protein specie. Use of narrow pH range (5-8) ampholytes is being made to further eliminate undesirable proteins from the crude extract. Enrichment of desired protein followed by IEF promises large yield.

Also available for viewing as POSTER NO. 24.

49

STRUCTURE FUNCTION STUDIES OF JASMONATE-INDUCED RIBOSOME-INACTIVATING PROTEIN, A POTENTIAL CANDIDATE TRANSGENE. **B. Chaudhry and J. Mundy**. Institute of Molecular Biology, Oster Farimagsgade 2A, 13533 Copennagen K, Denmark.

Jasmonate is implicated as a defensive hormonal signal. It induces several defence proteins in barley leaves. This work aims the analysis of Barley jasmonate responsive gene and candidate transgenes with potential for fungal protection. The inhibition of ribosome functions associated with depurination of 26S rRNA, characteristic of plant RIPs. The N-terminal region of a 60 kDa, jasmonate-induced protein of barley leaves (JIP60) is shown to be homologous to the catalytic domains of plant ribosome inactivating proteins (RIP). Western blotting of leaf extracts and in vitro reconstitution experiments indicate that JIP60 is synthesized as a precursor which is processed in vivo. this is in keeping with *in vitro* translation experiments indicating that a deletion derivative of the N-terminal region, but not the putative precursor, strongly inhibits protein synthesis on reticulocyte ribosomes but donot significantly inhibit on wheat germ ribosomes. This indicates that JIP60 is a novel ribosome-inactivating protein. These and other results suggest that JIP60 may be involved in plant defence. Characterization of structure function of Jip60 would be presented.

We have also reported that barley Type 1 ribosome inactivating protein Rip30 is capable of inhibiting the growth of fungi *in vitro* and in transgenic tobacco (Leah et al.1991 JBC 266, 1564-73, Logemann et al.1992 Biotechnology 10, 305-80). We have made constructions of vectors for expressing barley Rip30 under different monocot promoters of Rice chitinase and maize PR1 with HPT or BAR selectable markers. These vectors have been made available to groups working in transformation for crop plants. Their potential to enhance fungal protection would be discussed.

Also available for viewing as POSTER NO. 25.

50

DETECTION, PURIFICATION AND CHARACTERIZATION OF ANTIFUNGAL ACTIVITY AGAINST ASCOCHYTA RABIEI. **S. Maqbool**. National Center of Excellence in Molecular Biology, Thokar Niaz Baig, Canal Bank Road, Lahore-53700, Pakistan.

Tnis work aims to make a search for antifungal proteins that could be used in transgenic technology to breed fungal resistance in chickpea. As part of these studies, different bacteria were isolated from different sources and screened for antifungal activity against *A. rabiei*. Two bacterial isolates identified as *B. subtilis* and *B. cereus*, exhibited strong antifungal properties. Active fractions from *B. cereus* and *B. subtilis* were purified and characterized. *B. cereus* fraction was proteineous in nature while the other was non proteinous. Further studies on *B. cereus* fraction established that the antifungal fraction was due to a modified peptide. Further, identification and elucidation of its structure is in progress.

Further, different plant species were selected according to their antimicrobial and medicinal properties, and tested against *A. rabiei*. Activity was only observed in Garlic (*Allium Sativum*). Aqueous extract of garlic (*Allium Sativum*) exhibited antifungal activity, which was isolated and purified by using different chromatographic techniques. The active fraction is characterized as a small molecular weight sulfur containing organic compound. On further investigation, this compound is identified as *Allicin*.

Another aspect of this work was the study of different isolates of *A. rabiei* by fingerprinting for a reliable characterization of the genetic make up of *A. rabiei* that might help in the breeding resistance in chickpea. Data about fingerprinting of thirteen *A. rabiei* isolates will be presented.

Also available for viewing as POSTER NO. 26.

51

ROLE OF A. RABIEI VIRULENCE FACTOR IN BREEDING RESISTANCE TO CHICKPEA. **Z. Latif and A. A. Shahid**. National Centre of Excellence in Molecular Biology, University of the Punjab, Thoker Niaz Baig, Canal Bank Road, Lahore-53700, Pakistan.

This project aims towards a biochemical level of detection and characterization of the phytotoxins present in *A.rabiei* and study of the possible role of these phytotoxins in blight infected chickpea. Four different phytotoxic compounds as solanapyrones A, B, C & cytochalasin D were identified in nine isolates of *A.rabiei* collected from blight infected chickpea plants in Punjab Region. Variation in correlation coefficient was observed between the titer of phytotoxic compounds synthesized in the host plant consequent to fungal infection and the disease scale. Considerable variability within *A. rabiei* isolates for pathogenicity and virulence has been determined. Kinetic study of phytotoxins production during *in vitro* growth of various isolates of *A. rabiei* has determined a variable level of these compounds. Isolation, purification, characterization and possible role of these toxins in breeding resistance in chickpea would be discussed.

Also available for viewing as POSTER NO. 27.

52

IDENTIFICATION OF PATHOTYPES IN ASCOCHYTA RABIEI (PASS) LAB. THE CAUSE OF CHICKPEA BLIGHT IN PAKISTAN. F. Jamil, I. Haq, M. Sarwar and N. Bashir. Biological Chemistry Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan.

Investigations on pathogenic variability in *Ascochyta rabiei*, the causal organism of chickpea blight revealed great variation in pathogenicity of its various isolates. A number of isolates of *A. rabiei* were obtained during annual surveys of chickpea crop grown in various parts of the country from 1984-94. Isolates differing in cultural characters were selected for biological pathotyping. One hundred and twenty one selected isolates were studied for pathogenic variability using a differential host set, classified into eight pathogenic groups. Ont of 121 isolates tested so far, 2 were placed in group I, 7 in group II, 21 in group III. 32 in group IV, 40 in group V, 11 in group VI, 7 in group Vil and 1 in group VIII. Isolates of group I were highly virulent, and those of group VIII were least virulent.

The application of molecular marker techniques for genotyping (DNA fingerprinting) of isolates from each pathogenic group has been initiated which will supplement our present work on biological pathotyping and will enable us to clearly identify the physiological races/pathotypes in *A. rabiei* prevalent in Pakistan.

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UPTAKE, TRANSPORT AND METABOLISM OF TRICHLOROETHYLENE AND PENTACHLOROPHENOL BY NICOTIANA TABACUM. **S. Rashid and Z. Ahmed**. National Centre of Excellence in Molecular Biology, University of the Punjab, Thokar Niaz Baig, Canal Bank Road, Lahore-53700, Pakistan.

Tobacco (*N. tabacum*) seedlings when exposed for 24 hours in a hydroponic system, transport and metabolize trichloroethylene (TCE) and Pentachlorophenol (PCP). The TCE metabolites were found throughout the plant while most of the metabolites of throughout the plant while most of the metabolites of Pentachlorophenol were immobilized in the root tissue. Five different metabolites of TCE were estimated by HPLC. One predominant species was found in root, stem and leaf tissues of the extractable metabolites. Trichloroethanol has been identified as a major and free metabolite in root and leaves. There is an indication that Cytochrome P450 is involved in the degradation of trichloroethanol. It is predicted that degradation of TCE and PCP by tobacco may help to understand degradation of chemical contaminants by other plants. It is further added that tress like poplar may also be helpful to clean up soil and water contaminated with trichloroethylene and Pentachlorophenol.

Also available for viewing as POSTER NO. 32.

54

SETTING UP OF MUTAGENICITY/TOXICITY TESTS FOR ENVIRONMENTAL MONITORING. Q. M. Khan, Z. M. Khalid, K. A. Malik and A. Nasim. Environmental Biotechnology Division National Institute for Biotechnology and Genetic Engineering (NIBGE) P.O. Box 577. Jhang Road, Faisalabad, Pakistan.

Industrial and chemical processes produce substances toxic to the environment and human health. Occasionally such substances are simply introduced into environment without detoxification. For this reason, testing for the induction of DNA damage and for mutagenicity, using a variety of short-term tests, has become an accepted part of the toxicological evaluation of drugs, industrial intermediates, cosmetics, food and food additives, pesticides, biocides and industrial effluents etc. Monitoring the environment itself (e.g., air, water) are other activities which use tests based on the detection of DNA damage and mutation.

However, no single mutagenicity test is capable of detecting all mutagens so our overall approach (at NIBGE) is to establish a battery of "microbial tests" with *E. coli* , *Salmonella, Saccharomyces cereviciae* and *Shizosaccrhromyces pombe* to test for potential mutagenicity and carcinogenicity of test substances.

In the present study using Ames test more than 20 samples (dyes, effluent from various sections of textile industry, Cr compounds) were tested for their mutagenicity. The dyes like direct pink, reactive violet and reactive yellow were positive (mutagenic) while brilliant yellow was negative to mutagenicity testing strains TA98 and TA100. Effluent samples from printing and dyeing sections of textile industry appeared mutagenic to strains TA98, TA100 and WP101. Bio-treatment of textile effluents in general decreased mutagenicity. However, anaerobic treatment of dyeing effluent as well as aromatic dyes with a consortium of bacteria resulted in increased mutagenicity. Chromate salts are used in tanning industry. When tested, sodium chromate and potassium dichromate were positive to the Ames test. Higher concentrations of these salts (crystals in spot test) were even toxic for the test strains. As expected sodium azide and nitrosogunadine (MNNG) exhibited positive test, indicating reliability of the test.

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THE IMPACT OF AMBIENT AIR POLLUTION ON WHEAT IN THE PUNJAB, PAKISTAN. A. Wahid, R. Maggs[1], S.R.A. Shamsi, M.R. Ashmore[1], J.N.B. Bell[1]. Department of Botany, University Of The Punjab, Quaid-e-Azam Campus, Lahore, Pakistan. [1]Department of Biology, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berks. SL5 7PY. UK.

Increased industrialization and urbanization in recent years in many of the low-and middle-income countries of south-east Asia and the Pacific have meant corresponding increases in the anthropogenic emissions which have occurred largely unchecked. Little attempt has been made to determine the impact of such rapid change on agricultural systems.

Eight open-top chambers of the design used by Imperial College, London have been developed in the New Campus Botanical Garden, Punjab University, on the outskirts of Lahore. Four plots were ventilated with filtered air (FA) and four ventilated with unfiltered air (UFA). A further four unchambered open plots (OP) enable comparisons with field conditions.

The mean 6-h concentration of ozone over the growing season was 35.6 ppb, with an overall filtration efficiency of 85%. The ozone concentration increased from mean level of 27.0 ppb in December and January to 39.5 ppb during March and April. Uniike ozone, nitrogen dioxide remained virtually constant over the duration of the experiment with a mean of 23.3 ppb and filtration efficiency of 65%.

The experiment involved two locally grown wheat cultivars, Pak-81 and Chak-86. Development of Pak-81 below showed increased tiller production in FA plots compared to both UFA and OP treatments. Senescence of main stem leaves were also delayed in FA plots. Highly significant differences ($P < 0.001$) were found in total grain weight per plant for both cultivars in UFA, with reduction of 46% and 34.8% for Pak-81 and Chak-86 respectively, when compared to FA. Further reductions of 4% and 12.5% were seen in OP yield.

There is some evidence that ozone is the major pollutant responsible for these large reductions in the yield. The effects of filtration on vegetative development began at about the time ozone levels began to increase. European and American studies have reported significant yield reductions of wheat as a result of ozone at 7-h mean concentrations of 40-50 ppb, a concentration only slightly above that seen in the later part of the experiment although the yield reductions are lower than those in our experiment.

Also available for viewing as POSTER NO. 33.

56

PRODUCTION OF CUT FLOWERS THROUGH MICROPROPAGATION. **I. A. Nasir and H. Afrasiab.** Centre of Excellence in Molecular Biology, Thokar Niaz Baig, Canal Bank Road, Lahore-53700, Pakistan.

Tissue culture of different varieties of Rose, Amaryllis and Gladiolus was initiated. Callus formation has been obtained from leaf discs of rose in MS medium containing 2,4-D 4mg/l, kinetin 1mg/l and 5% coconut milk. Twin scales ($1Cm^2$) of Amaryllis when cultured in MS medium containing BAP 2mg/l and IBA 8mg/l gave plantlets. These plantlets were multiplied and transferred to soil. Corm pieces ($1Cm^2$) of Gladiolus were cultured on MS medium containing BA 0.07mg/l and NAA 0.02mg/l. Plantlet formation was observed and small corms developed after 10-12 weeks which will be transferred to soil. Work is in progress to obtain plants with unique characteristics as a result of somaclonal variation.

57

AGROBACTERIUM TUMEFACIENS: HOST RANGE, HOST SPECIFICITY AND T-DNA EXPRESSION. **S. H. A. Qazi and S. Hasnain**. Department of Botany, University of the Punjab, Lahore. Pakistan.

Agrobacterium tumefaciens was isolated from young tumors of *Erythrina indica* (EI2, EI3) *Acacia modesta* wall. (AM1), *Cedrus deodara Roxb.* ex Lamb. (CD1) and *Melia azedarach* L. (MA5). All isolates caused tumorigenesis on explant as well as intact plant. The host range experiments revealed that all the isolates could induce tumor on *Brassica oleracea* L. var. *botrytis* L., *Capsicum annuum* L. var. *grossum* L., *Cicer arietinum* L., *Cucumis melo* L., *Medicago falcata* L., *Momordica charantia* L., *Petunia alba*, *Pisum sativum* L., *Raphanus sativus* L. They showed different responses on *Glycine soya* L., *Oryza sativa* L., and *Zea mays* L. None of the isolates could produce any tumorigenecity response on *Triticum aestivum* L. Co-cultivation experiments were performed with callus cultures of *Brassica oleracea* L. var. *capitata* L. and transfer of T-DNA was demonstrated by auxin independent growth of these calli. Auxin content of these calli were significantly higher as compared to the control (not infected) calli growing on auxin supplemented medium. Results of co-cultivation experiments revealed that transformation was achieved in all the isolates.

58

FLUX CONTROL ANALYSIS OF PRIMARY NITROGEN ASSIMILATION IN MAIZE MESOPHYLL CHLOROPLASTS. M. Ashraf and M. J. Emes[1]. Department of Chemistry, Islamia University, Bahawalpur, Pakistan. [1]School of Biological Sciences, University of Manchester, Oxford Road, Manchester, M13 9PT. UK.

Which enzyme is rate-limiting to the flux of a pathway? Why is this important if we are about to engineer a strain for the purpose of increasing the flux through one of the metabolic pathways? Flux control theory provides the answer that which enzyme should be engineered for the particular metabolite. This theory, which can be applicable to any system, has been applied to the primary nitrogen assimilation in plants.

The main objective of work has been to determine the importance of each component of nitrite reduction and amino acid synthesis in controlling assimilatory nitrogen flux in chloroplasts. Flux control coefficients of nitrite reductase (NiR), glutamine synthetase (GS) glutamate synthase (GOGAT) and phosphorylation were determined by varying nitrogen availability to the plant and using specific inhibitors of enzyme reaction.

Mesophyll chloroplasts were isolated from leaves of *Zea mays* plants grown at different nitrogen concentrations. Using inhibitors of GS, GOGAT and components of photosynthetic electron transport/phosphorylation, the control coefficients of different steps were determined. GS was found to have a high control coefficient for glutamate synthesis, although in the presence of glutamine the contribution of GOGAT became significant. NiR was found to exert minimal control of glutamate synthesis though its activity influenced the balance of control between GS and GOGAT.

Cyclic photophosphorylation was found to have a high control coefficient for glutamate synthesis which increased in chloroplasts of plants grown at low nitrogen availability. ATP concentrations were strongly correlated with flux to glutamate indicating a high control strength for this metabolite. These are the first results indicating the quantitative importance of different components of primary nitrogen assimilation in a C_4 plant.

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LOCAL PRODUCTION OF MOLECULAR BIOLOGY PRODUCTS. **R. Mushtaq, S. Choudhry, Z. Rahman, E. Ahsan and V. Nadeem**. Centre of Excellence in Molecular Biology, University of the Punjab, Thokar Niaz Baig, Canal Bank Road, Lahore-53700, Pakistan.

In view of recent international developments in the area of genetic engineering and biotechnology various research institutes in Pakistan have taken initiatives to carry out basic as well as applied research. The Centre for Applied Molecular Biology (CAMB) aims at providing specialized research products of the highest purity to the scientific community. CAMB has made a modest beginning and started to share some of its end products with other research organizations and has educational institutions in Pakistan, in addition to fulfilling its own needs.

Eleven commonly used restriction enzymes (*Bam*HI, *Bgl*I, *Bgl*II, *Bli*RI, *Bte*I, *Cfr*A4I, *Eco*RI, *Kpn*I, *Pae*AI, *Sau*MI, *Ste*I), cloning vehicles and substrate DNAs (pBR322, pUC19, M13mp18RF, T7 Phage DNA, Lambda Phage DNA, pGEM-3Z, Bluescript, M13mp18 single stranded), standard size marker (Lambda *Hin*dIII Digest) and custom oligonucleotides are available at no profit basis. A survey is being conducted through distribution of free sample kits to various research establishments within the country to estimate "market demands". The free package includes selected restriction enzymes, their reaction buffers, substrate DNA and size standard marker alongwith a recent catalog and technical data sheets. Production of these materials at CAMB will help to overcome difficulties in the acquisition of such critical laboratory materials and ensures ready availability thus contributing to promote teaching and research in genetic engineering and biotechnology within the country.

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ALTERED STABILITY OF HYBRIL PLASMID BY DELETING DNA SEGMENTS.

S. Hasnain and S. K. Sherwani. Department of Botany, University of the Punjab, Lahore Pakistan.

Hybrid plasmid pSH1012, which carry *B. subtilis* genomic segment in *Escherichia coli* vector, exhibit stability both at low as well as at high copy number. This plasmid hybridise with *pyr* gene, of *B. subtilis*. While rest of the hybrid plasmids carrying *pyr* gene also indicated by hybridisation, display instability at high copy number. It reflects that different DNA segments may have impact on plasmid stability. To study the effects of deletions on the stability, deletion derivatives of pSH1012 were constructed. Genetic mapping of pyr genes on these deletion derivatives was mapped and plasmid segregational instability of these deletion derivatives were studied. Different DNA deletions altered the plasmid stability.

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THE STUDY OF INTERACTION OF THE STAPHYLOCOCCAL BETA-LACTAMASE-CONFERRING PLASMID PI9789::TN552 WITH THE HOST CHROMOSOME. M. Sohail and K. Dyke[1]. Biomedical and Genetic Engineering Division, Dr.A.Q. Khan Labotatories, G-9/1, Islamabad-44000, Pakistan, [1]Microbiology, University of Oxford, Oxford OX1 3QU, UK.

A derivate of the plasmid pI9789::Tn552 that is temperature-sensitive for replication (pSI) can integrate into the chromosome of the *Staphylococcus aureus* strains by a process which can be catalysed by the site-specific recombinase BinR. The integration involves chromosomal DNA that has homology to part of pI9789::Tn552. Details of this interaction are presented. In a different strain of *S. aureus* (1054), a co-integrate is formed between the plasmid pSI and a large cryptic plasmid p1054. Some investigations on the nature of this co-integrate are described.

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MOLECULAR EPIDEMIOLOGY OF CLINICAL ISOLATES OF SHIGELLA FLEXNERI AND THE USE OF POLYMERSE CHAIN REACTION TO DETERMINE THEIR RELATEDNESS. K. Sultana, M. Sohail & C. A. Batt[1]. Department of Biology, Quaid-i-Azam University, Islamabad, Pakistan. [1]Institute of Food Science, Cornell University, Ithaca, USA.

Investigations were caried out on *S. flexneri,* isolated from faecal samples of diarrhoeal cases presented during a period of three months at a local hospital in Islamabad. Fifteen pathogens were identified usig routine procedures. Antibiotic resistance analysis to eleven antimicrobial drugs showed the presence of mutiple and different resistance phenotypes in all isolates. Plasmid profile analysis showed that all strains also exhibited a varied plasmid population. Sub-typing by the protein profile analysis using SDS-PAGE method of whole cells as well as outer membranes showed that these isolates fell into two groups. A powerful approach using synthetic random primers and Polymerase Chain Reaction (PCR) indicated genetic relatedness amongst the isolates dividing these into two classes, which however do not represent the same grouping pattern as shown by the protein sub-typing. The usefulness of these typing methods in the charaterisation of clinical isolates is discussed.

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ALTERATION OF GLUTATHIONE TRANSFERASE ISOENZYME PATTERN IN CHEMICALLY INDUCED RAT HEPATOCYTES. **M. K. Tahir**. Department of Chemistry, University of Azad Jammu and Kashmir, Muzaffarabad (A.K.), Pakistan.

The glutathione transferase constitutes a family of detoxication enzymes that catalyze the reaction of glutathione with a number of electrophilic compounds including certain mutagens and carcinogens. Multiple forms have been identified in different tissues of man, mouse and rat. The different cytosolic isoenzymes have been grouped into three classes-Alpha, Mu and Pi, on the basis of multiple criteria including immuno-crossreactivity and amino acid sequence data.

The distribution of three classes has been studied in normal and transformed cells. Remarkable changes in glutathione transferase expression have been observed during chemical carcinogenesis. Elevated levels of class Pi trasferase have been observed in transformed cells. In a rat hepatoma cell line, 95% of the total transferase activity towards 1-chloro-2,4-dinitrobenzene (universal substrate for glutathione transferases)was accounted for by class Pi enzyme. This enzyme is hardly detectable in normal hepatocytes. Class Alpha and class Mu enzymes, that constitute a high percentage of glutathione transferase contents in normal liver were down regulated to almost undetectable concentrations. The results indicate a possible role of glutathione transferase at different stages of cancer.

64

DEVELOPMENT OF B. THURINGIENSIS BASED INSECTICIDES. G. Rubi, E. Khan and Z. Ahmad. Center of Excellence in Molecular Biology, University of the Punjab, Thokar Niaz Baig, Canal Bank Road, Lahore-53700, Pakistan.

Pest control in Pakistan is largely dependent on chemical insecticides and currently no biocontrol agent is in use for agricultural pests or malarial vectors. The aim is to exploit the larvicidal potential of *B. thuringiensis* to develop low cost Bt based microbial products for the control of different insect pests and vectors. In persuit of this objective, two isolates of *B. thuringiensis* separated from a local habitat and showing high entomocidal activity were studied for their growth and retention of toxic activity in cheap industrial waste materials. Heat resistant spore counts in some of the by-product media tested were found to be higher than in modified G medium. Corn steep liquor based media gave the highest spore counts (10^{15}/ml) and retained the virulence of the isolates when tested in laboratory biotoxicity assays. Shake flask experiments were successfully scaled upto 300 liter fermenters. Preliminary small scale potato field trials against *Heliothis armigera* and pond trials against *Anopheles stephensi* clearly indicate the potential of *B. thuringiensis* for commercial use as a bilogical control agent in Pakistan.

65

IN VITRO PROPAGATION OF BOUGAINVILLEA (B. SPECTABILIS) THROUGH SHOOT APEX CULTURE. **M. A. Javed and S. Hassan**. Nuclear Institute for Food and Agriculture P.O. Box No. 446 Peshawar, Pakistan.

Shoot apices of *Bougainvillea spectabilis* cv. Texas Dawn were excised, surface sterilized and cultured on MS modified medium containing different concentration and combination of plant growth regulators/hormones for shoot development, shoot proliferation/and root induction. Maximum shoot development was observed when BAP 0.25 + NAA 0.25mg/l were added to the medium. Combination and concentrations of NAA 0.1 + BAP 0.25 and NAA 0.1 +BAP 0.5 also gave good shoot development response. The treatment containing NAA 0.1 + BAP 2.0 + Glutamine 250mg/l induced significantly maximum number of multiple shoots. Shoot proliferation was also observed better on NAA 0.1 + BAP 0.5 + Glutamine 250mg/l combination. Significantly higher percentage, (70%) of rooting took place when IBA 5.0 + NAA 5.0 were added to the medium. Medium showed good rooting percentage when added 2.5 and 5.0 mg/l of IAA alone. The system will provide a means of rapid clonal propagation in future.

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SCREENING OF BACILLUS THURINGIENSIS ISOLATES AGAINST PESTS IN THE FAMILY TENEBRIONIDAE AND APHIDIDAE. **K. Malik and R. Anwer**. Centre of Excellence in Molecular Biology, University of the Punjab, Thokar Niaz Baig, Canal Bank Road, Lahore-53700, Pakistan.

Tribolium castaneum (coleoptera: Tenebrionidae) is a serious pest of stored grains, flour and flour products. Aphids (Hemiptera:Aphididae) are plant sucking insects which are responsible for two types of damage to the plants. Firstly, direct damage, resulting from search for food which induce plant deformation. Secondly, indirect damage caused by the transmission and spread of various phytopathogens.

The biotoxicity assay procedure for *Tribolium castaneum* and aphids has been established. *Tribolium castaneum* were maintained at $30 \pm 1^{O}C$ with relative humidity of $60 \pm 5\%$, reared on a diet containing wheat flour, semolina, yeast extract in jars covered with muslin cloth. Different concentrations of Bt endotoxin separated from various Bt strains were mixed in the above diet and third instar larvae were released in the diet vials, kept at constant temperature and humidity. Of Bt isolates checked against larvae of *Tribolium castaneum* two isolates gave 50% mortality at 300ng/mg and 800ng/mg respectively.

For aphids, Bt crystal protein was mixed with 20% sucrose and the mixture was used as diet. This diet solution was placed between two stretched layers of parafilm wrapped on petri plates which were lined with moist filter paper containing the aphids. Several local isolates were examined against aphids (*Aphis gosypii* or *B. brassicae*), out of which one gave 90% mortality (1000ug/ml). Screening work is in progress.

67

FUMONISIN B1, B2 AND MONIFORMIN INDUCED LYMPHOCYTE CYTOTOXICITY DETECTION THROUGH MTT-BIOASSAY. T. Javed. Nuclear Institute for Agriculture and Biology, P.O. Box. 128, Faisalabad, Pakistan.

Peripheral blood lymphocytes were isolated from broiler chicks that had ingested feed amended with FUMONISIN B1 (FBI), FUMONISIN B2 (FB2) and MONILIFORMIN. Lymphocyte viability was determined for birds that had started on amended rations at day 1 and day 7 at three levels of mycotoxins, ranging from 61-546 ppm FB1, 14-94 ppm FB2 and 66-367 ppm MONILIFORMIN. Reduction of the tetrazolium salt, MTT [3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyl tetrazolium bromide], to yield MTT formazan was used to assess cell viability, based on mitochondrial metabolic activity. Cytotoxic effects were observed; chicks that started on amended feed at day 1 of age were more susceptible than those that started at day 7. Abnormal erythrocytes resembling early stages of erythroblasts were observed in peripheral blood from test chicks. Some red cells were spindle-shaped with one end pointed and the other rounded, or with both ends pointed. Red cells appeared to be undergoing mitosis. The test may be applied to observe the immunomodulation of the lymphocytes and macrophages.

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MOLECULAR BIOLOGY AND BIOTECHNOLOGY APPLICATION FOR THE CONSERVATION AND PROPAGATION OF NATIVE COLOMBIAN PLANT SPECIES. G. A. Gongora, M. S. Carrizosa, G. Cancino, C. Serrano. Plant Biology Unit, Biology Department, Universidad Javeriana, Bogota, Colombia.

Methods are being developed for germplasm conservation and micropropagation of species of the Passiflora genus, fruit-bearing vines native to northern South America, and Cedrela montana, a native Colombian angiosperm tree of economic potential. In order to evaluate genetic stability of conserved and micropropagated material of passiflora, techniques have been developed for isolation and purification of chloroplasts, and isolation of ctDNA. Detection of polymorphic restriction fragments are used to differentiate Passiflora species and evaluate genetic changes during germplasm conservation and micropropagation. C. montana explants from juvenile plants (3-6 months old) and aseptically-germinated plantlets have ben established *in vitro*. Axillary bud development requires no growth regulators and elongated shoots can then be rooted with 1.3 uM naphtalenacetic acid (NAA). Other explants, including those derived from adult trees, are being evaluated for their organogenic potential. The conservation of germplasm and propagation of selected material of *C. montana* and Passiflora species offers good economic potential to local Colombian economies.

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CONCURRENT SESSIONS (TIME 1400-1830 DATED 11.04.1995)

ADMINISTRATION BLOCK, CEMB

SESSIONS	SESSION ROOM
Session VI: DNA Replication.	A
Session VII: Gene Cloning	B
Session VIII: Plant Transformation	C
Session IX: Molecular Diagnostics	D
Session X: Fungal Plant Interaction	E
Session XI: Environmental Biotechnology	F

TRANSPORT FACILITY AVAILABLE FOR SYMPOSIUM PARTICIPANTS

SUNDAY, APRIL 09 & 10, 1995

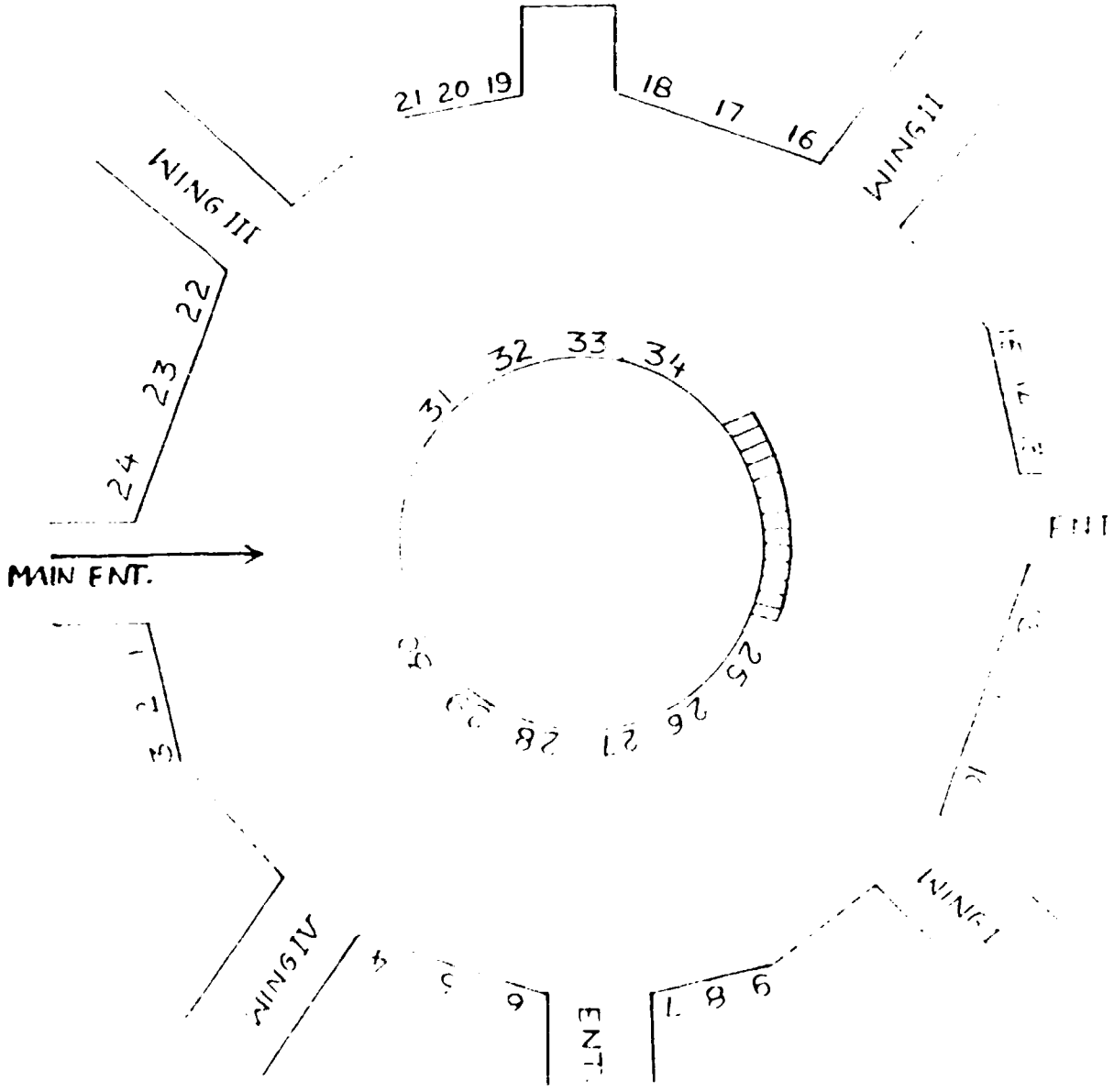
7:00 a.m.	:	Departure from CEMB
7:10 a.m.	:	University Guest House
7:20 a.m.	:	STC Center, University
7:30 a.m.	:	Shalimar Hotel, Liberty
7:40 a.m.	:	University Grants Commission
7:55 a.m.	:	Avari Renaissance

MONDAY, APRIL 11, 1995

12:30 p.m.	:	Departure from UGC
12:35 p.m.	:	Shalimar Hotel, Liberty
12:40 p.m.	:	STC Center, University
12:50 p.m.	:	Arrival at CEMB

The transport will be available along the same route at the end of the last session of the day.

POSTER DISPLAY SITES IN THE CENTRAL AREA OF LAB BLOCK AT CEMB



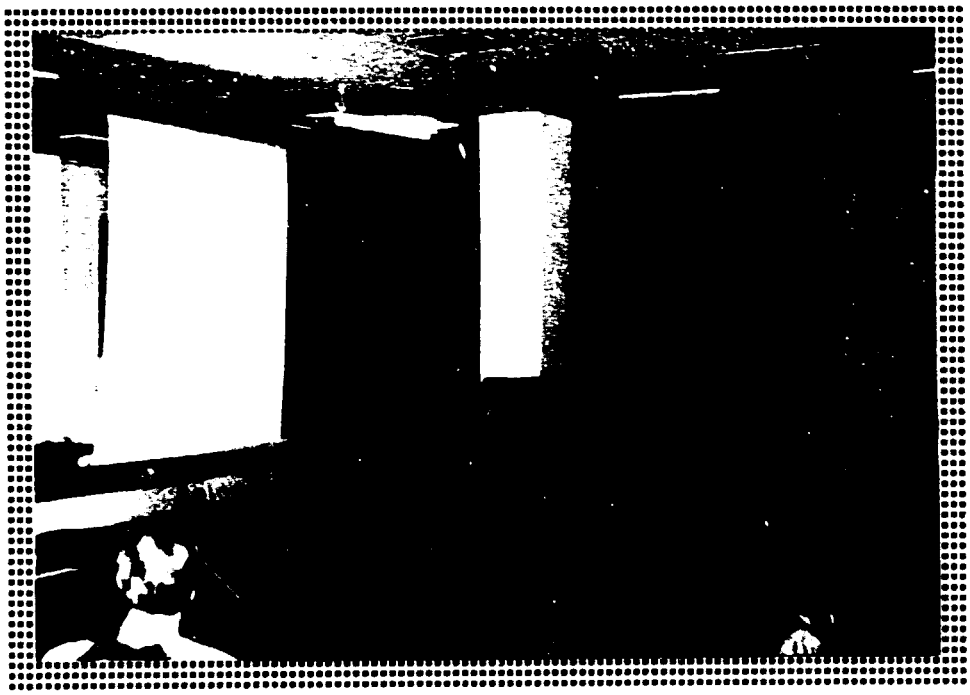
Professor Lawrence Grossman presenting his plenary lecture. In the background is Professor Richard J. Roberts (Nobel Laureate), Chairing the Session.

Professor Stuart Linn, Professor, BMB Div. University of California, presenting his plenary lecture.

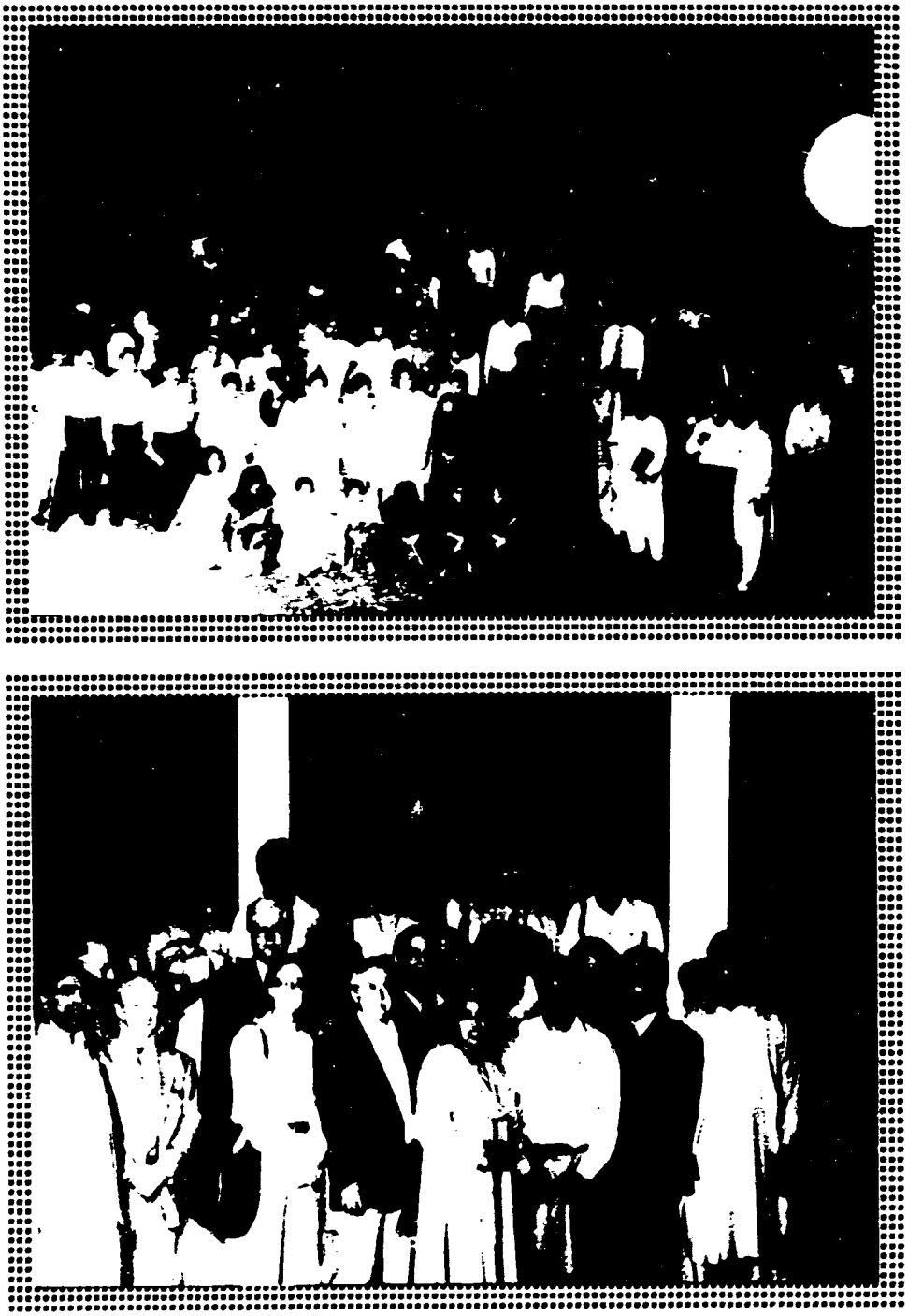
A Glimpse of the audience bewitched by an excellent plenary lecture on DNA Methylases by Prof. Richard J. Roberts (Nobel Laureate).

Professor G. Khush (India) presenting his plenary lecture on crop production in the developing world and crop improvement strategies. Seen in the background are Professor H.O. Smith (Nobel Laureate) and Professor S.H. Mujtaba Naqvi, Director General, Nuclear Institute for Agriculture and Biology, Chairing the session.

Professor S. Riazuddin presenting his plenary lecture on Bt as an environmentally friendly agent for controlling plant pests. Professor H.O. Smith (Nobel Laureate) and Professor S.H. Mujtaba Naqvi Director General, Nuclear Institute for Agriculture and Biology are Chairman and Co-chairman of the session.



Professor Jan E. Leach, Department of Plant Pathology, Kansas State University, presenting her plenary lecture on induction of defense responses in rice. Professor H.O. Smith (Nobel Laureate) is Chairing the Session.



Symposium participants grouped together for a photograph just before the concluding ceremony.