International Conference on the Applications of Molecular Biology in Medicine and Agriculture

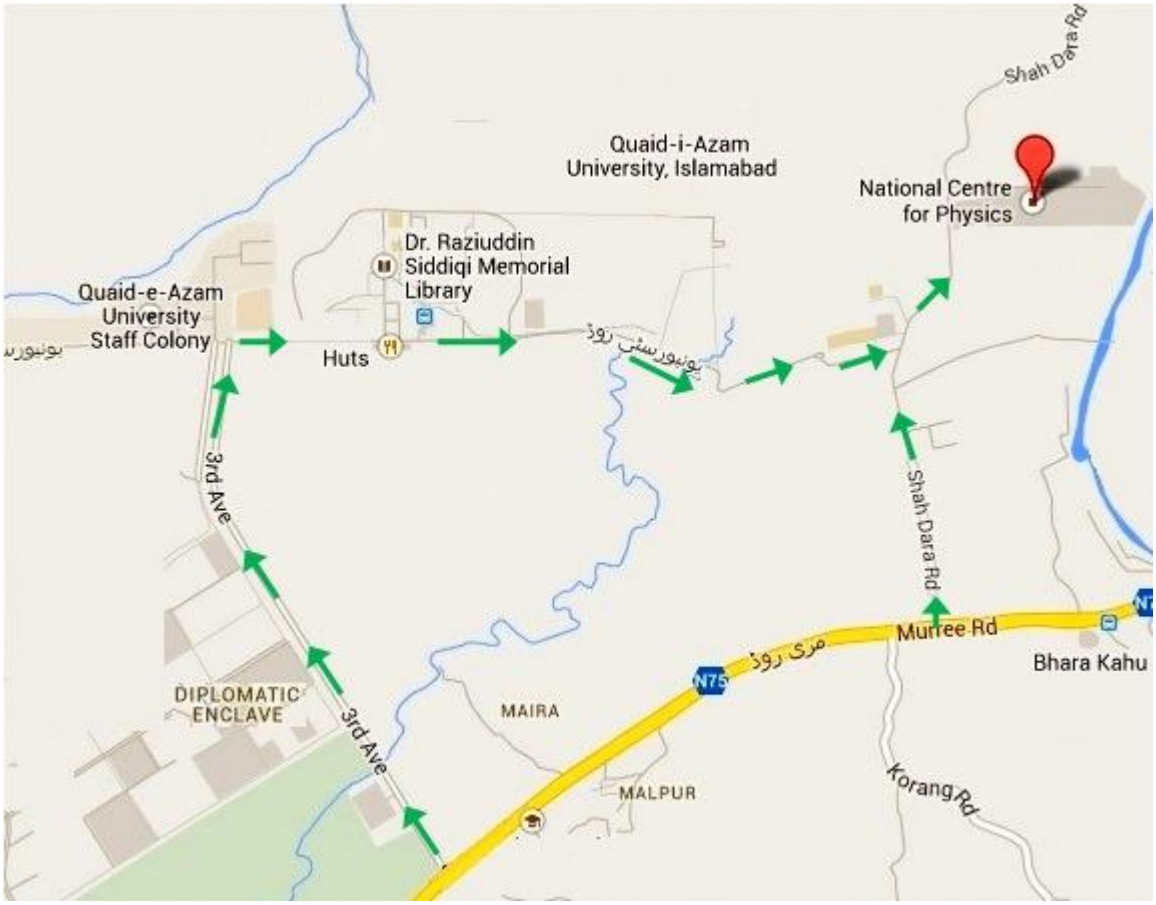
August 20-22, 2013

Abstract Book

Department of Biochemistry, Quaid-i-Azam University, Islamabad
Biochemists Association Quaid-i-Azam University, Islamabad (BAQI)
ISESCO Women in Science Chair – In collaboration with
The Islamic Educational, Scientific and Cultural Organization (ISESCO)
Pakistan National Commission for UNESCO & ISESCO

Conference Venue

National Centre for Physics
Quaid-i-Azam University Campus
Shahdrah Valley Road
Islamabad, Pakistan



Directions: From Murree road, take turn either on **3rd avenue** or **Shahdara road** and follow the arrows to reach the venue

Contents

Vice Chancellor's Message

It gives me immense pleasure in recording this message for "International Conference on the Applications of Molecular Biology in Medicine and Agriculture". My pleasure is three-fold since I am the Vice-Chancellor of this institution, I am also the Patron-in-Chief of BAQI and being Biochemist, I share the same subject.

Biochemistry/Molecular Biology is a very exciting subject and is considered extremely important in understanding the other areas of Biology. The techniques developed by the Biochemists and Molecular Biologists have been extremely useful for research and applications in all spheres of Biology and applied sciences. This conference will help to promote scientific culture and mutual relations among the individuals for future perspectives.

I wish BAQI and students associated with it, a success in promoting this important science to play its due role. Quaid-i-Azam University enjoys a distinct position among the various academic circles of our country and is the top ranked institution. We strongly believe in the leadership of the students in co-curricular and extracurricular activities and therefore the University administration strongly supports such positive moves for the better learning opportunities. As young scientists, BAQI members have a role to play in the socio-economic uplift of the country. We have ambitious plans for this University and our main focus is on producing best quality graduates for the country in particular and the world in general. One more expectation that I have from BAQI is to promote entrepreneurship among the young Biochemists and catalytic leadership in youth.

Prof. Dr. Masoom Yasinzai
Patron-in-Chief BAQI
Vice-Chancellor
Quaid-i-Azam University
Islamabad, Pakistan

Dean's Address

It gives me enormous gratification to welcome the invitees of International Conference on Applications of Molecular Biology in Medicine and Agriculture. I am deeply indebted to the Chief Guest for gracing this occasion. I am confident that under his able leadership, guidance and patronage, we would be able to pursue the objective of promoting cooperation amongst all represented countries in scientific and technological fields vigorously in order to achieve greater progress, prosperity and self-reliance.

With the rapid increase in world population, we are entering a new century that will be dominated by research and development of applications of Biochemistry/ Molecular Biology. Being a Plant scientist, I believe that these predictions are true. However, it needs to be understood that development is a multifaceted process, and a number of factors must dove-tail together before economic growth and progress can occur.

As Dean, Faculty of Biological Sciences and Professor of Plant Sciences, I deem that this conference is covering diverse areas of Molecular Biology (medicinal plants, drug discovery, model organism for drug delivery). Faculty of Biological Sciences is playing important role for development and research in various fields with regard to Biochemistry and Molecular Biology, Animal Sciences, Biotechnology, Microbiology, Plant Sciences, Bioinformatics, Environmental Science and Pharmacy.

The Faculty has a well-established herbarium meeting the requirement for plant systematics, taxonomy and plant biodiversity related research activities. The plants and data available in this facility will soon become online and this national treasurer can be used for national and international studies. At present the herbarium has a collection of nearly 155,000 specimens from 250 families. Herbarium of Quaid-i-Azam University is the largest herbarium in the country and second largest in South Asia in terms of plant specimens and identification services. With National Herbarium (rich collection of plants from different parts of Pakistan including some rare plants and many endemics) and Botanical garden of Aromatic and Medicinal Plants, Faculty of Biological sciences is one of leading research establishment of Pakistan contributing to national and international ranking of Quaid-i-Azam University.

The Botanic Garden is an important extension of the Faculty of Biological Sciences. It will not only be an on-site recreational facility with social and community benefits but its establishment will also provide important research facilities for ex-situ conservation of

medicinal and aromatic plants, seed bank, in-vitro storage and DNA bank etc. The project also has non-commercial research perspective as it will benefit fields such as taxonomy, genetics, physiology, anatomy, ecology, seed-banking and horticulture.

I strongly believe that international event like this will lead to development of national and international collaboration of students as well as faculty for uplift of Pakistan's economy. I value the contribution of BAQI for organizing this event and hope that they continue these valiant efforts for betterment of Quaid-i-Azam University.

Prof. Dr. Asghari Bano
Dean
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad

Welcome Note

We extend our warmest welcome to all the participants of International Conference on Applications of Molecular Biology in Medicine and Agriculture (August 20-22, 2013) organized by Biochemists Association Quaid-i-Azam University, Islamabad (BAQI), Department of Biochemistry, Quaid-i-Azam University, Islamabad and ISESCO Women in Science Chair. It gives us great pleasure to have research scholars and friends in Islamabad from all over Pakistan and abroad. Our common interest in the field of Molecular Biology brought us together today and it will continue in future at broader spectrum. This conference will provide a platform to researchers to share current research trends and learn about new ideas and techniques. This forum will also provide a way to establish networks to support future research in this exciting area.

The primary goal of the conference will be to promote scientific information interchange among the students, faculty and researchers. This multidisciplinary conference will feature plenary talks by Keynote speakers and oral as well as poster presentations.

We are very excited to host this integrative event at our university and hope that you will enjoy all the sessions.

Prof. Dr. Bushra Mirza
Patron BAQI & Chairperson
Department of Biochemistry
Quaid-i-Azam University
Islamabad

Acknowledgement Note

It is a great pleasure to welcome all the students, researchers and scientists to our conference. The main aim of creating this multidisciplinary scientific environment is to develop a high performance and interactive scientific platform for students, academic colleagues, and renowned scientists which could help in establishing international scientific network.

We all are looking forward to the keynote lectures from our invited speakers and give a special thanks to the foreign speakers for flying all the way to Pakistan and dedicating so much time for the event. We proudly announce that there will be prizes for best oral and poster presentation which will be awarded in the closing ceremony at the end of conference.

We would especially like to thank Prof. Dr. Masoom Yasinzai (Patron-in-Chief BAQI), Prof. Dr. Salman A Malik (Founder Patron BAQI), Prof. Dr. Bushra Mirza (Patron BAQI) and Prof. Dr. Wasim Ahmad (Conference Advisor) for their expertise and support during the organization of this conference. We would also like to thank all young scientists, researchers and faculty members for submitting their abstracts and look forward to strong networking and collaboration. The efforts and hard work of BAQI team, especially Raja Hussain Ali, President BAQI is praiseworthy.

Finally, we would like to thank Higher Education Commission Pakistan, Pakistan Academy of Sciences and ISESCO Women in Science Chair for being our main sponsors.

We are looking forward to a successful and inspiring event!

Dr. Mariam Anees
Conference Secretary
Department of Biochemistry
Quaid-i-Azam University
Islamabad

About BAQI

On behalf of the Biochemists Association Quaid-i-Azam University, Islamabad (BAQI), I welcome you all to the International Conference on the Applications of Molecular Biology in Medicine and Agriculture.

Biochemists Association Quaid-i-Azam University Islamabad (BAQI) is a registered association of Biochemists and Molecular Biologists that was established under the umbrella of Department of Biochemistry Quaid-i-Azam University in 2011. It aims to promote scientific culture by organizing seminars, conferences, workshops, job fairs, and is committed to provide knowledge based economic output to country. BAQI is also trying to bridge the gap by connecting Biochemistry and Molecular Biology professionals working in different sectors so that collective efforts may carve a path for young researchers and students which could help in their career building.

We are very much excited to have such great international and national scientists and look forward to benefit from their experience and scientific insight. We are thankful to all our participants and hope that our guests will participate in future BAQI events as well.

Raja Hussain Ali
President BAQI
Ph.D. Scholar

BAQI Cabinet

President	Raja Hussain Ali
Vice President	Asif Khan
General Secretary	Sohail Ahmed
Joint Secretary	Sidra Bukhari
Treasurer	Hanif Ullah Khan
M.Phil. Coordinator	Wajid Ameen
M.Sc. Coordinator	Ghulam Murtaza

BAQI owes a special acknowledgement to Younus Khan, Azfar Ali Bajwa and Muhammad Umair for their enthusiastic support in organizing the event.

Organizing Committee

Patron in Chief:	Prof. Dr. Masoom Yasinzai Vice Chancellor Quaid-i-Azam University Islamabad
Patron/Coordinator:	Prof. Dr. Bushra Mirza – Chairperson
Conference Advisor:	Prof. Dr. Salman Akbar Malik Prof. Dr. Wasim Ahmad
Secretary:	Dr. Mariam Anees – Assistant Professor
Treasurer:	Dr. Muhammad Rashid Khan – Associate Professor
Facilitation/Logistics:	Dr. Qamar Javed – Associate Professor Dr. Muhammad Ansar – Associate Professor Dr. Samina Shakeel – Associate Professor Dr. Iram Murtaza – Assistant Professor Dr. Aneesa Sultan – Assistant Professor Dr. Muhammad Tahir Waheed – Assistant Professor Dr. Rubab Satti – Assistant Professor

Program

Day 1 – 20th August 2013

10:00am – 12:30pm	Registration
12:30pm – 01:30pm	Inauguration
01:30pm – 02:30pm	Lunch and prayer break
1st Technical Session	**Medicinal Plants and Evolutionary Biology**
02:30pm – 03:00pm	**Keynote Lecture**: Some Australian Medicinal Plants – The Beginning of an Odyssey. *Kerr PG, Charles Sturt University, Australia*
03:00pm – 03:15pm	Antimicrobial Potential of some Important Medicinal Plants from Qarshi Herbarium. *Jaffar Q, Altaf A, Hussain A & Jamil A, University of Agriculture, Faisalabad*
03:15pm – 03:30pm	Cytotoxic Compounds from *Fagonia cretica L*. Exerts p53 Dependent and Independent Apoptosis via DNA Damage in Human Breast Cancer Cells. *Saleem S, Kondratyuk TP, Chang LC, Mirza B & Pezzuto JM, Quaid-i-Azam University, Islamabad*
03:30pm – 03:45pm	Identification, Conservation and Phylogenetic Analysis of Medicinal Plants of Northern Areas of Pakistan through DNA Barcoding. *Tahir A, Altaf A & Jamil A, University of Agriculture, Faisalabad*
03:45pm – 04:15pm	**Keynote Lecture**: Molecular Basis of Calyx Inflation; A Postfloral Innovation. *Khan MR and Ali GM, National Agricultural Research Center, Islamabad*
04:15pm – 04:45pm	**Poster Session – I**

Day 2 – 21st August 2013

2nd Technical Session	**Tools in Modern Research**
09:30am – 10:00am	**Keynote Lecture**: Science on the Edge: Controversies and Breaking Paradigms in Modern Research. *Ali A, Canadian Intellectual Property Office & University of Windsor, Canada*
10:00am – 10:15am	Targeting Drug Delivery of Anticancer Drug Conjugated with Magnetic Nanoparticles through Transferrin Receptors. *Samra ZQ, University of the Punjab, Lahore*
10:15am – 10:45am	**Keynote Lecture**: Experience of Introducing Prenatal Diagnosis for Genetic Disorders in Pakistan. *Ahmed S, Genetics Resource center, Rawalpindi*
10:45am – 11:30am	**Poster Session – II** & Tea

3rd Technical Session	**Molecular Diagnostics and Signal Transduction**
11:30am – 12:00pm	**Keynote Lecture**: Molecular Diagnostics: Potentials and Limitations. *Jamil N and Bilal R, Biological Research Center, Karachi*
12:00pm – 12:15pm	A Novel Mechanism in Cardiac Remodeling of Gender Based KO Mouse Model. *Haroon J, Hussain S, Foureaux G, Martins AS, Ferreira AJ, Santos RAS, Bader M, Reis AM & Javed Q, Quaid-i-Azam University, Islamabad*
12:15pm – 12:30pm	Identification of Bone Morphogenetic Proteins (BMP) as Key Instructive Factor for Human Epidermal Langerhans Cell Differentiation and Proliferation. *Yasmin N, Bauer T, Seyerl M, Schuster C, Koeffel R, Stoeckl J, Elbe-Buerger A & Strobl H, Medical University of Vienna, Austria*
12:30pm – 12:45pm	Rapid Method to Diagnose the Isoniazid, Ethambutol and Multi Drug Resistant Tuberculosis. *Rehman A, Malik SA & Khanum A, Quaid-i-Azam University, Islamabad*
12:45pm – 01:00pm	ATP Binding Cassette (ABC) Transporters as Drug Target. *Parveen Z, Stockner T, Bentele C, Pferschy S, Kraupp M, Freissmuth M, Ecker FG & Chiba P, Abdul Wali Khan University, Mardan*
01:00pm – 01:30pm	**Keynote Lecture**: Depression: Diagnosis, Prevalence, Treatment and Importance of CYP2D6 Allele in Karachiates. *Farooq AD, University of Karachi, Karachi*
01:30pm – 02:30pm	Lunch and prayer break
4th Technical Session	**Environmental and Agricultural Sciences**
02:30pm – 03:00pm	**Keynote Lecture**: Ecological Changes as Indicators of Climate Change *Mermut AR, University of Saskatchewan, Canada; Harran University, Turkey*
03:00pm – 03:15pm	Assessment of B-Amylase Production Potential in Aspergillus fumigates on Agricultural Waste. *Sajid A, Asad MJ, Mahmood RT, Gulfraz M, Asghar M, Hussain S, Zafar M, University of Agriculture, Faisalabad*
03:15pm – 03:30pm	Microbial Diversity and Biopolymer Potential of Salt-Affected Land. *Ashraf M, Bousserrhine N, Abbad S, Mahmood K, Latif F & Kamal GM, Nuclear Institute for Agriculture and Biology, Faisalabad*
03:30pm – 03:45pm	Isolation and Characterization of Rhizosphere Bacteria of Rice Paddy Field Based on 16S rRNA Sequence Analysis. *Sarwar A, Nassem M, Sajjid I, Riaz Rabaila, Shahwani N & Ahmad N, Balochistan University of Information Technology, Engineering and Management Sciences, Quetta*
03:45pm – 04:00pm	Larvicidal and Insecticidal Activity of Streptomyces Isolates of Salt Range, Pakistan. *Anwar S & Sajid I, University of the Punjab, Lahore*

Day 3 – 22nd August 2013

5th Technical Session	**Genetics and Hereditary Diseases**
09:30am – 10:00am	**Keynote Lecture**: A Drosophila model of Hereditary Sensory and Autonomic Neuropathy Type 1. *Sweeney ST, University of York, United Kingdom*
10:00am – 10:15am	FZD6 Encoding the Wnt Receptor Frizzled 6 is Mutated in Autosomal Recessive Nail Dysplasia. *Naz G, Pasternack SM, Perrin C, Mattheisen M, Refke M, Khan S, Gul A, Simons M, Ahmad W & Betz RC, Quaid-i-Azam University,*
10:15am – 10:30am	*Islamabad*Novel Mutation in SLC4A11 in Two Pakistani Families Affected with Congenital Hereditary Endothelial Dystrophy (CHED2). *Kaul H, Ullah MI, Suman M & Khan Z, University of Health Sciences, Lahore*
10:30am – 11:00am	**Keynote Lecture**: Autosomal Recessive Primary Microcephaly (MCPH): From Molecular Genetics to Diagnostic Applications in Pakistan. *Hassan MJ, Shifa College of Medicine, Islamabad*
11:00am – 12:00pm	Tea
12:00pm – 01:00pm	Concluding Session (Certificate/ Award Distribution)
01:00pm – 03:00pm	Lunch and See Off to Delegates/Guests

Keynote Lectures by Foreign Speakers

Philip Grey Kerr, Ph.D.

School of Biomedical Sciences
Charles Sturt University
Wagga Wagga, New South Wales
Australia

Some Australian Medicinal Plants – The Beginning of an Odyssey

This presentation is a semi-historical, illustrated account of my introduction to the study of the medicinal plants of Australia. For many years, the anecdotal reports of medicinal use of plants by the indigenous people of Australia had been collected but were ignored and/or forgotten. Occasionally, the story entails suspense and intrigue. In large part it has been the renaissance of interest in plants as potential medicinal agents that has stimulated my interest in the phenomenal chemical variation in plants, as well as their worldwide use as medicines. Effectively, it is pharmacy in the Western (European) tradition that has come around full-circle... back to its roots.

The phytochemical focus of the lecture is on plants from the families Goodeniaceae, Gyrostemonaceae and Myrtaceae. The major biological foci are antibiotics, cancer, diabetes and neurological disorders. These are an outgrowth of my PhD studies on Scaevolaspinescens (Goodeniaceae), a plant which still holds my attention and that of a number of my Australian colleagues. In the presentation, I will outline some of the techniques used and results obtained, many of which remain to be disseminated more widely than conferences and theses held in university libraries.

Sean Thomas Sweeney, Ph.D.

Department of Biology
University of York
York, United Kingdom

A Drosophila Model of Hereditary Sensory and Autonomic Neuropathy Type 1

Hereditary Sensory and Autonomic Neuropathy type 1, (HSAN1) caused by dominant inheritable mutations in the gene encoding subunit one of the enzyme Serine Palmitoyltransferase (Spt1), leads to a pathology of distal limb sensory loss and ulceration. Spt performs the first step in de novo sphingolipid synthesis by the condensation of serine with palmitoyl-CoA suggesting an essential function for these lipids in nervous system function.

We use the causative mutation in HSAN1, coupled with the genetic manipulation available in the Drosophila model system to explore the role of sphingolipids in neuronal development and function. We use the larval neuromuscular junction synapse to explore the role of sphingolipids in synapse formation and growth and show that both pre- and post-synaptic sphingolipid synthesis is required for development of this model synaptic structure. The sensory nervous system is then targeted to produce a Drosophila model of HSAN1. HSAN1 model larvae show reduced pain sensation and a reduction in exocytosis in sensory dendrites. The transgenic introduction of the ER – Golgi trafficking mediator, Rab1, provides a phenotypic rescue of reduced pain sensation suggesting that sphingolipids play a role in ER – Golgi trafficking. This observation therefore suggests that the predominant pathology of HSAN1 is due to a sphingolipid-depletion linked ER – Golgi trafficking defect. Other studies have suggested that an incorporation of alanine, as opposed to serine, into the nascent sphingolipid is responsible for the pathology. Here, we show that the phenotype can be rectified by feeding serine, while alanine feeding induces a more sever phenotype.

Adnan Ali, Ph.D.

[1]*Canadian Intellectual Property Office*
Gatineau, QC
[2]*University of Windsor,*
Windsor, Canada

Science on the Edge: Controversies and Breaking Paradigms in Modern Research

Within the eukaryotic cells, there are numerous regulatory networks that regulate the ultimate fate of the cells. These signaling networks comprise a number of different protein products whose activity is itself regulated by other specific signaling molecules from within the cells as well as in response to any external stimuli from outside the cells. These signaling mechanisms are responsible for the cellular behavior and activities in eukaryotic cells. Various signaling cascades can be initiated using proper inducers or inhibitors that may trigger these cellular activities instantly in vitro. Through the visualization of such processes, using proper tools, the cells can be examined for any physiological changes in response to external stimuli by exposing the cells to toxic or other chemical compounds. Such experiments using modern approaches allow scientists to understand the mechanism of signal transduction within the cells as well as the possible roles of signaling molecules in various cellular activities. Further, the significance of the protection of intellectual property rights will also be discussed in conjunction with the use of modern technology in contemporary science.

Ahmet Ruhi Mermut, Ph.D.

Professor of Soils
[1]University of Saskatchewan
Saskatoon SK, Canada
[2]University of Harran
Harran, Turkey

Ecological Changes as Indicators of Climate Change

There is a general worldwide consensus to study the complicated interactions between the nature and all living matters in order to adapt, or mitigate various natural and man-made changes within the biosphere-geosphre-society systems. The soil is one of the most important natural resource for producing food and fiber for mankind and naturally feed for animals for sustainability on earth. In addition to the physical support of life, the soils have many other important functions, such as atmospheric, hydrospheric, and lithospheric. Soils function not only as a water purification-nutrient-life media but also as redistributors and regulators of most of the important fluxes of matter and energy, especially carbon. Soil changes takes place in short, medium and long term and this is excellarated by ever-increasing desire to utilize by man. The changes in soils may be gradual, rapid, or even catastrophic. Intensive works have been carried out and are now going on about the biosphere-geosphere and lithosphere at global scale and on the importance of describing and understanding changes in soils for the functioning of the whole earth system. These human induced rapid chances require human responses.

Global Carbon Cycle is one of the basic and fundamental problems for mankind. Soils play a very effective role on the global terrestrial carbon stocks and cycle. There are, however, many problems to deal with large uncertainty that we have today. Some of these are: 1) inadequate estimates of the biomass amounts and variations characteristic of various biotic communities and their soils, 2) inability to precisely measure historic and present changes in land use and deforestation, and 3) poor information about carbon dynamics between pedosphere, hydrosphere and atmosphere. The current uncertainties in estimation of carbon stocks and variations obtained from very limited actual soil samples and release of carbon from the various terrestrial systems, including agriculture. Measurements of below ground biomass will require large expenditure and manpower for actual field studies. We must focus our attention the cycle of carbon, to establish sustainable life on earth and possibly find out ways and means to deal with global climate change.

Keynote Lectures by National Speakers

Ahsana Dar Farooq, Ph.D.

International Center for Chemical and Biological Sciences
University of Karachi
Karachi, Pakistan

Depression: Diagnosis, Prevalence, Treatment and Importance of CYP2D6 Allele in Karachiates

Depression is a global phenomenon affecting children, adults and aged people but its prevalence varies among different countries. About 10% of people suffer from depression worldwide and according to WHO estimates it will be 2nd most common healthcare challenge by 2020 and needs serious consideration. Although, both environmental factors (particularly stress) and genetics are accountable for its expression but its severity can be reduced after proper diagnosis, treatment and its management. There are many antidepressants available in the market but due to low efficacy and undesirable side effects better and well tolerated alternates are required. Additionally, some patients are either responders or non-responders to the available antidepressants that might increase the chances of drug toxicity.

At International Center for Chemical and Biological Sciences, University of Karachi, Pakistan in collaboration with chemists, antidepressants derived from natural products and/or synthetic compounds were evaluated in rodents using behavioral, biochemical and immunohistochemical studies. Areca catechu nut ethanolic extract, aqueous fraction, particularly saponin-rich fraction significantly reduced immobility time in rats and elevated levels of neurotransmitters (5-hydroxytryptamine and noradrenaline) in their hippocampus. Likewise, Opuntiadillenii cladodes methanol extract and its aqueous fraction in addition to reduction in immobility time in rodents also inhibited monoamine oxidase-A activity suggesting that both plants possesses antidepressant like properties. Among synthetic compounds, 2-phenylethyl alcohol, isoamylphenylacetate and N-(4-nitrophenyl)-N'-(1'-phenylethyl) urea displayed maximum inhibition of 43%, 37% and 90%, respectively, suggesting they possess potent antidepressant-like activity.

In collaboration with the psychiatrists, the frequency of depressive symptoms on Hamilton Depression rating scale (HDRS) was assessed. Our study demonstrated that depressed mood, anxiety psychic and somatic were evident in ~80% of the depressed patients. However, agitation, loss of interest in work and activities, insomnia and diurnal variation were evident in about 70% of individuals. Among various symptoms of HDRS insomnia, agitation, suicidal thoughts, somatic symptoms and diurnal variation were higher in female

patients. After 2 weeks of the antidepressant treatment with paroxetine demonstrated maximum reduction (45%), in HDRS score whereas, flouxetine showed minimum reduction (21%). This reduction was accompanied by 31% (2 weeks) and 43% (6 weeks) decline in their MAO-B activity in platelets with paroxetine being most effective.

A variable drug metabolizing ability of individuals has been noticed worldwide that is governed by a polymorphic CYP2D6 locus, thus any antidepressant that works fairly quickly and in a large percentage of patients would be highly desirable. Keeping this in mind allelic variants *1 (normal responder), *4 (poor responder) and *10 (intermediate responder) in normal and depressed subjects from Karachi population were analyzed. The frequencies of CYP2D6*1 (wild type allele) was highest in normal subjects (57%) as compared to depressed (32%) individuals whereas, CYP2D6*4 null allele was more prevalent in depressed (13%) subjects. The mutant allele CYP2D6*10 was about 10% more frequent in depressed (54%) than in the normal individuals. These results suggests that CYP2D6*10 allele is predominantly present in Karachiates that represents intermediates drug responders and the clinicians while prescribing the antidepressant should consider it and adjust the doses accordingly.

Thus proper diagnosis of depression along with appropriate use of antidepressants doses will minimize the sufferings of the depressed patients.

Muhammad Jawad Hassan, Ph.D.

Shifa College of Medicine
Islamabad, Pakistan

Autosomal Recessive Primary Microcephaly (MCPH): From Molecular Genetics to Diagnostic Applications in Pakistan

Autosomal Recessive Primary Microcephaly (MCPH) is a rare disorder of neurogenic mitosis characterized by reduced head circumference at birth with variable degree of mental retardation. In MCPH patients, brain size reduced to almost one-third of its original volume due to reduced number of generated cerebral cortical neurons during embryonic neurogensis. So far, ten genetic loci (MCPH1-10) for this condition have been mapped with all ten corresponding genes identified from different World populations. Contribution of ASPM (MCPH5) and WDR62 (MCPH2) genes mutations in MCPH is more than 50%.

By and large, primary microcephaly patients are phenotypically indistinguishable, however, recent studies in patients with mutations in at least some of the microcephaly genes showed a broader clinical and/or cellular phenotype. It has been proposed that mutations in MCPH genes can cause the disease phenotype by disturbing: 1) orientation of mitotic spindles, 2) chromosome condensation mechanism during embryonic neurogenesis, 3) DNA damage response signaling, 4) transcriptional regulations and microtubule dynamics, 5) certain unknown centrosomal mechanisms that control the number of neurons generated by neural precursor cells. Recent discoveries of mammalian models for MCPH have open up horizons for researchers to add more knowledge regarding the etiology and pathophysiology of MCPH.

High incidence of MCPH in Pakistani population reflects the most probable involvement of consanguinity. In fact, presence of different ethnic/linguistic backgrounds within Pakistani population and high levels of consanguinity among these groups give us an opportunity to establish ethnic group based mutation database. This will certainly help in diagnostics and will reduce the cost. On the other hand, genetic heterogeneity of the disorder (10 genes so far and many other coming...) is a barrier for establishment of diagnostic facilities for affected families in particular and for general population in common.

Aim of the talk is to address some basic detail about the disease and to discuss some suggestions for establishment of diagnostic facilities for this disorder. Genetic counseling and clinical management through carrier detection/prenatal diagnosis in MCPH families should be the focus of research in this field as this can help reducing the incidence of this autosomal recessive disorder.

Muhammad Ramzan Khan, Ph.D.

National Institute for Genomics and Advanced Biotechnology
National Agricultural Research Centre
Islamabad, Pakistan

Molecular Bases of Calyx Inflation; a Postfloral Innovation

(Muhammad Ramzan Khan and Ghulam Muhammad Ali)*

One of the greatest evolutionary innovation, the true flower occasionally manifests variability in the architecture such a alteration in symmetry, fused sepals, petals and leaf like sepal just to name a few. Of these, calyx inflation culminating in Solanaecae has recently fascinated evolutionary biologists. One of the fundamental questions is whether a regulatory circuit exist in plants to control floral calyx identity under selective pressures in exhibiting this unique evolutionary innovation. This is indeed the case according to studies that attempt to entangle the complexity associated with calyx encapsulation. The origin of inflated calyx is associated with the heterotopic expression of the MADS box gene 2 from Physalisfloridana (MPF2) in floral organs and interactions of MPF2-MPF3 as well as action of plant hormones. Functions of the Withaniahomeologs after genome duplication, diverged during evolution and both the coding and cis-regulatory elements contributed equally for functional dispersal between paralogs (MPF2-like-A and MPF2-like-B). At the C-terminal of these duplicates indels, single nucleotide polymorphisms, and exonization of intron were detected. Furthermore, evolution of MPF2-like genes entailed degenerative mutations is the core promoter CArG-box and auxin response factor (ARF) binding element in the large 1st intron. Besides, modular action of cis-elements added to genetic and phenotypic variations in the Solanaceous plants and enhanced the potential of natural selection for the adaptive evolution of morphological novelty of calyx inflation.

Rakhshanda Bilal, Ph.D.

Defence Science and Technology Organization
Biological Research Centre
Karachi, Pakistan

Molecular Diagnostics: Potentials and Limitations

(Nadia Jamil and Rakhshanda Bilal)*

Over the past several years, the development and application of molecular diagnostic techniques has initiated a revolution in the diagnosis and monitoring of infectious diseases. Nucleic acid techniques are making increasing inroads into detection and identification of pathogens hence diagnosis of diseases. These techniques are helpful in detecting an organism directly from clinical samples, without the need for culture, be it un-culturable, fastidious or pathogenic/hazardous microorganisms. Other important progress includes the determination of viral load and the direct detection of genes or gene mutations responsible for drug resistance of pathogenic organisms.
Increased use of automation and user-friendly software make these technologies more extensively applicable. Molecular methods have now progressed beyond identification to detect antimicrobial resistance genes and provide public health information such as strain characterization by genotyping. Genotypic variation of same pathogen creating different serotypes can be identified by various techniques, in the same way a pathogenic gene hidden in a commensal organism can be detected easily.

As DNA chip technology improves, the ability to test for multiple organisms will become easier whether it is in field or lab. Altogether, the detection of infectious agents at molecular level represents a true amalgamation of clinical chemistry and clinical microbiology techniques. Costs of molecular methods are decreasing such that the role of molecular methods will further increase. In the coming years, molecular diagnostics will continue to be of critical importance to public health worldwide and it will facilitate the detection and characterization of disease, as well as monitoring of the drug response, and will assist in the identification of genetic modifiers and disease susceptibility. However, there are major hurdles to overcome, such as which test to employ, the choice of technology and equipment, and issues such as cost-effectiveness, accuracy, reproducibility and intellectual property. Moreover there are some limitations of the molecular techniques like false positive and false negatives, which can be address in one way or other. The talk will focus on the potentials and limitations of molecular methods and some methods to overcome these problems.

Maj Gen (R) Suhaib Ahmed, HI (M)

MBBS, MCPS, FCPS, PhD
Genetics Resource Centre
Rawalpindi, Pakistan

Experience of Introducing Prenatal Diagnosis for Genetic Disorders in Pakistan

Genetic disorders constitute approximately 5% of the burden of diseases in Pakistan. Thalassemia is the commonest single gene disorder with an approximate carrier rate is 5% and each year over 5000 children are born with thalassemia major. The other common genetic disorders include trisomy 21 and a large variety of autosomal recessive and X - linked disorders. Management of genetic disorders is difficult as well as expensive. Therefore the affected children pose severe health and psycho social burden for their families.

Prenatal diagnosis is offered to the couples who either have an affected child or in whom other risk factors are identified. The first step in the diagnosis is fetal sampling that is usually done after 10 weeks of gestation by ultrasound guided trans-abdominal chorionic villus sampling. The fetal sample is processed in the lab for either a DNA based testing or by culture for chromosome analysis.

A clinical service for prenatal diagnosis of thalassemia and some other common genetic disorders was introduced for the first time in May 1994. At present there are at least five centers in the country where this facility is available. During the last couple of decades over 10,000 prenatal diagnoses for various genetic disorders have been done at these centers. Thalassemia is the commonest indication followed by Trisomy 21 and Duchenne Muscular Dystrophy etc. Prenatal diagnosis is technically feasible and is gradually gaining wide acceptance amongst all socio-economic and religious classes. There is a consensus amongst all religious scholars in Pakistan that Islam permits termination of pregnancy for a genetic disorder provided it is done before 17 weeks of gestation. Over 90% of the couples terminated the pregnancy when they were told about the fetus being affected by a genetic disorder. High cost of the health care, rather than the religious beliefs, was the commonest reason for failing to terminate the pregnancy when required.

Lack of awareness, high cost of test and difficulty in accessing the facility were the commonest reasons for not using prenatal diagnosis when it was required. Religious beliefs, contrary to the popular belief, were the least common reason for avoiding prenatal diagnosis.

Oral Presentations

OP-01

Identification, Conservation and Phylogenetic Analysis of Medicinal Plants of Northern Areas of Pakistan through DNA Barcoding

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DNA barcoding is a new concept which aims to provide rapid, accurate and automatic species identification by using a standardized DNA region as a tag. In developing countries, medicinal plants are still used for healthcare as they possess biologically active molecules for the development of modified derivatives with enhanced activity and reduced toxicity. Even crude extracts of medicinal plants may be used as medicaments which can be easily adulterated because there are no methods to identify the processed and damaged plant materials. Northern areas of Pakistan are rich in plant biodiversity which includes numerous medicinal plant species. Many of these species are on the verge of extinction due to insufficient management and wild collection. Conservation and sustainable use of medicinal plants require accurate and rapid identification. Here, medicinal plants were collected from different areas of Swat, morphologically identified and preserved as voucher specimens for future references. matK, rbcL, trnH-psbA regions of chloroplast DNA and ITS region of nuclear genome were amplified by Direct Plant PCR using specific primers for each region. Amplicons were purified and sequenced which served as barcodes and markers for identifying the medicinal plants. These barcodes were then further utilised for phylogenetic analysis of selected medicinal plants by using various bioinformatics tools. Obtained barcodes is a step towards rapid and convenient identification of dry and damaged samples of medicinal plants. Moreover, phylogenetic analysis on the basis of these barcodes will further authenticate the position of studied plants in the tree of life.

OP-02

Assessment of β-Amylase Production Potential of Aspergillus fumigatus on Agricultural Waste

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β-amylase is a maltogenic hydrolytic enzyme that perform the hydrolysis of starch into maltose. Microorganisms grow on starchy material produce it for the hydrolysis of substrate. In current study Aspergillus fumigatus, isolated locally was used for the production of β-amylase on rice straw. Its production was optimized by employing various cultural and nutritional parameters. β-amylase was partially purified and characterized to achieve its maximum enzymatic activity. Maximum β-amylase was obtained after 72 hrs of Solid State Fermentation at 500C temperature, pH 5 and in the presence of 70 % moisture level. Its production was further increased with the addition of 0.2 % glucose, 0.2% peptone and 0.3% tween-20. Tween-80 decreased the production of β-amylase. It was partially purified by 40% ammonium sulfate by precipitation and 5 % silica gel column. The most suitable pH and temperature for β-amylase were 4.8 and 550C. Calcium, magnesium and zinc metal ions have positive effect on β-amylase activity. Partially purified β-amylase at optimum conditions can be used for industrial purpose.

OP-03

Isolation and Characterization of Rhizosphere Bacteria of Rice Paddy Field Based on 16S rRNA Sequence Analysis

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Rice addresses a key global staple food to one third population of the world. Because of rapidly increasing population, the crop sustainability must be considered to keep pace with population growth. Due to the potential role of microbial community to plant sustainability and production, the role of the rhizosphere has gained enormous importance in the last few decades. This study is aimed at identification of bacteria isolated from soil samples collected from the rhizosphere of rice fields of district Jaffarabad, which belongs to the major rice growing area of Pakistan. On the basis morphological and physiological properties, and 16S rRNA gene sequence analysis six strains B9, B15, B34, B40, B52 and B53 were identified as genus Bacillus whereas isolate B12 as genus Staphylococcus. Isolates B9, B40 and B53 showed 99% similarity to Bacillus thuringiensis, Bacillus anthracis and Bacillus endophyticus respectively. Discrepancy was observed for B15, B34 and B52 for sequence analysis based on BLAST and SeqMatch alignment. The isolate B12 showed 99% similarity to Staphylococcus aureus. Our findings indicate that instead of resorting only to biochemical tests, the validation further through 16S rRNA can result in a more objective view of the structural & functional composition of soil bacterial community.

OP-04

Rapid Method to Diagnose the Isoniazid, Ethambutol and Multidrug Resistant Tuberculosis

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Tuberculosis (TB) is one of the major health troubles worldwide. Emergence of multi drug resistant tuberculosis is another threat coming up. Therefore, present study aimed to molecularly characterize the isoniazid and ethambutol resistance associated mutations in genes like katG, inhA regulatory region, emb 306 and emb 497 in local Mycobacterium tuberculosis isolates using PCR-single stranded conformational polymorphism, melt curve analysis and sequencing. KatG 315 mutation (AGC to ACC) has the highest mutation frequency with 87% in multidrug resistant (MDR), 57% in only isoniazid (INH) resistant samples, 50% in isoniazid and streptomycin (INH+STR) resistant and 50% in EMB and other drug resistant samples. While, percentage frequency of C to T mutation in inhA regulatory region was highest with 28.5% in only INH resistant sample, while MDR samples showed 22% inhA mutation frequency and 25% in INH+STR resistant samples. Further it was found that ethambutol (EMB) resistant samples carry a mutation at codon 306 (ATG to ATA). Frequency of emb 306 mutation is found to be only 15% in MDR samples, 100% in EMB and other drug resistant samples and 75% in only EMB resistant samples while mutation at codon 497 of EmbB was not found in this study. Comparison of results of three techniques used in this study showed that in terms of sensitivity and specificity of PCR-SSCP for the detection of isoniazid and EMB resistant samples was 81% and 100% respectively. While, melt curve analysis showed 86% and 100% respectively. Results of melt curve analysis were consistent with those of DNA sequencing. Hence, melt curve analysis can be effectively used as a simple, cost effective and rapid method for primary screening of katG315, inhA regulatory regions and embB306 mutations which found to be strong potential markers that occur in isoniazid and ethambutol-resistant and multidrug resistant M. tb isolates.

OP-05

FZD6 Encoding the Wnt Receptor Frizzled 6 is Mutated in Autosomal-Recessive Nail Dysplasia

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Informatics, International Islamic University, Islamabad, Pakistan; [5]Center for Biological Systems Analysis, Renal Division, University Hospital Freiburg, Germany

BACKGROUND: Isolated nail dysplasia is rare and has been reported in only a small number of families. OBJECTIVES: To describe and characterize two Pakistani families with an autosomal-recessive inherited nail dysplasia. METHODS: Genome-wide linkage analysis; mutation screening of candidate genes by Sanger sequencing; cloning of FZD6 and protein analyses; immunohistochemistry. RESULTS: We mapped this genodermatosis to chromosome 8q22.3, and identified a homozygous nonsense mutation c.1750G>T (p.E584X) in the frizzled 6 (FZD6) gene in all affected individuals. Immunohistochemical analyses in nail sections from healthy individuals revealed strong expression of FZD6 in the ventral nail matrix and a less pronounced expression of FZD6 in the nail bed. CONCLUSIONS: FZD6 belongs to a family of proteins that serve as receptors in Wnt signalling pathways, and has been shown to act as a negative regulator of the canonical Wnt/β-catenin signalling cascade and a positive regulator of the noncanonical Wnt or planar cell polarity pathway. The present results therefore suggest that FZD6 plays a pivotal role in the growth and guidance of the nail plate in humans by acting as a molecular switch between different Wnt pathways. Previous studies have identified mutations in the RSPO4 and LMX1B components of the Wnt pathway in patients with the hypoplastic nail disorders anonychia and nail-patella syndrome, respectively. Only recently, FZD6 mutations were identified in isolated nail dysplasia. The present results emphasize the important role of the Wnt pathways in nail development and increase understanding of Wnt-mediated developmental events in general.

OP-06

Novel Mutation in SLC4A11 in Two Pakistani Families Affected with Congenital Hereditary Endothelial Dystrophy (CHED2)

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Background: Investigation of molecular defect in autosomal recessive congenital hereditary dystrophy (CHED2) will help to identify the carrier of the disease. It can also become the foundation for mutation based prenatal genetic testing and hence could help the genetic diagnosis in families at risk for recurrence of disease. Objective: To identify the genetic defect in two families affected with autosomal recessive congenital hereditary dystrophy (CHED2). Materials and Methods: Two consanguineous families designated as CH01 and CH02 with autosomal recessive congenital hereditary endothelial dystrophy (CHED2) were recruited to participate in the study. Clinical evaluation of patients by trained ophthalmologists demonstrated that no other allied anomaly was segregating with CHED

phenotype, thus excluding syndromic mode of inheritance. Blood samples from families affected with CHED2 were collected from the patients and unaffected participants. Genomic DNA was isolated. Initially, linkage analysis using microsatellite markers was carried out to confirm the linkage to SLC4A11 gene, previously reported to be implicated in the pathology of the disease. Later on, sequencing was carried out to find the pathogenic mutation in the enrolled families. Identified mutation was further confirmed by typing 50 ethnically matched normal control samples. Results: The results of linkage analysis indicated the putative linkage to SLAC4A11 gene, located at the CHED2 locus on chromosome 20p13-p12 in both families. Mutational analysis revealed a novel homozygous mutation c.52106A>C (p.E702A) in the affected members of both the families. Pathogenicity of the identified mutation was confirmed by using bioinformatics tools as well as by sequencing 50 ethnically matched controls. Haplotype analysis of both the families showed that the affected members carry the same haplotype, thereby indicating the common ancestral mutation. Conclusions and Relevance: This study reports a novel mutation (c.52106A>C) in SLC4A11 gene segregating with diseased haplotype in two consanguineous Pakistani families.

OP-07

A Novel Mechanism in Cardiac Remodeling of Gender Based KO Mouse Model

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Genetic deletion of the Ang-(1-7)/Mas receptor leads to progressive cardiac changes in FVB/N mice on gender basis. The study presented here indicates a new gender based dimension of Ang-(1-7)/Mas in cardio-protection mechanism. We examined the expression levels of interleukins and natriuretic peptides in Ang-(1-7)/Mas knockout female (KF) and male mice (KM) as well as littermate controls (WF and KF). RT-PCR and immunohistochemical (IHC) analyses were performed, relating initial morphological, and histological findings. There were significantly increased left ventricular (LV) cardiomyocyte nuclei as well as high natriuretic peptides, and interleukins expression levels indicating LV hyperplasia in KM. Confirming our gender based model of cardiac hypertrophy and hyperplasia, KF had significantly smaller LV cardiomyocytes nuclei, and natriuretic peptides expression reflecting a significantly increased LV cardiomyocyte diameter, and interleukins expression compared to KM. Consequently, together these findings point toward a key pathologic role of the interleukin cytokines in the myocardium and identify

significant natriuretic peptides as a unique strategy to prevent functional LV myocardium after cardiac hypertrophy and hyperplasia inception following Ang-(1-7)/Mas ablation in FVB/N mouse.

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OP-08

Microbial Diversity and Biopolymer Potential of a Salt-Affected Land

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Microbes and the microbial processes are vital component of ecosystem functioning and integrity. The sustainability of both terrestrial and the aquatic habitats thus depends upon and is reflected by their microbial niches. Biotic and abiotic environmental stresses and the anthropogenic activities, however, disrupt the balance and put microbial harmony of the systems at stake. High soluble salts are responsible for loss of biological activities and productivity of the salt-affected lands. The gravity of the problem worsens further as the good quality irrigation water in the salt-affected areas is not available or it is brackish and contaminated. Use of low quality irrigation waters is perilous to both the environment and the human life. Microbial diversity, biopolymer potential and Arsenic resistant bacteria of soil and irrigation water resources of a salt-affected land were studied using molecular, biochemical, chemical and microbiological techniques. DGGE (Denaturing Gradient Gel Electrophoresis) analysis of PCR amplified 16S rDNA showed variability in phylo-genetic clustering, and the microbial diversity of three types of saline, saline-sodic and sodic soils and the brackish irrigation waters of the salt-affected land. Arsenic resistant bacteria were more abundant and prevalent in brackish and polluted irrigation waters than the salt-affected soils. The biopolymers produced by exo-polysaccharides (EPS) producing biofilm bacteria isolated from three types of soils ranged from 7.4-26.5%. The research findings suggested the salt-affected soils as a valuable source of the microbial biopolymers and the gene pool for industrial, environmental and biotechnological applications. Moreover, the study evokes the need to explore role of the enzyme levan sucrase in environmental implications especially the C-sequestration in terrestrial and the aquatic habitats.

OP-09

Identification of Bone Morphogenetic Proteins (BMP) as Key Instructive Factor for Human Epidermal Langerhans Cell Differentiation and Proliferation

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Human Langerhans cell (LC) precursors populate the epidermis early during prenatal development and thereafter undergo massive proliferation. The prototypic anti-proliferative cytokine TGF-β1 is required for LC differentiation from human CD34+ hematopoietic progenitor cells and blood monocytes in vitro. Similarly, TGF-βI deficiency results in LC loss in vivo. However, immunohistology studies revealed that human LC niches in early prenatal epidermis and adult basal (germinal) keratinocyte layers lack detectable TGF-β1. Here we demonstrated that these LC niches express high levels of BMP-7 and that BMP-7 induces LC differentiation and proliferation by activating the BMP type-I receptor ALK3 in the absence of canonical TGF-βI-ALK5 signaling. In turn, TGF-βl-induced in vitro LC differentiation is mediated via ALK3; however, co-induction of ALK5 diminished LC generation. Therefore, selective ALK3 signaling by BMP-7 allows exceedingly high LC yields. Within epidermis, BMP-7 shows an inverse expression pattern relative to TGF-β1, the latter induced in suprabasal layers and upregulated in outer layers. We observed that TGF-β1 inhibits microbial activation of BMP-7-generated LCs. Therefore, TGFβl in suprabasal/outer epidermal layers might inhibit LC activation, which in turn may secure LC network maintenance.

OP-10

Antimicrobial Potential of Some Important Medicinal Plants from Qarshi Herbarium

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Medicinal plants have a key role in the health care of people worldwide. Medicinal plants have a great potential for the treatment of various infectious diseases. The present study was conducted to investigate the antimicrobial assay based screening of important medicinal plants from Qarshi herbarium such as Ricinus communis, Cichorium intybus, Brassica napus, Plantago ovata, Foeniculum vulgare, Ocimum basilicum and Linum usitatissimum. The crude protein extracts of seeds showed significant activity against four bacterial species (Staphylococcus aureus, pasteurella multocida, Escherichia coli and

Bacillus subtillus) and four fungal species (Trichoderma harizanum, Ganoderma lucidum, Fusarium solani and Alternaria alternate). The extracts of Cichorium intybus and Ricinus communis showed maximum antifungal activity against Fusarium solani with the largest zones of inhibition. Such achievements would provide the opportunity to utilize the medicinal plants as a new source for production of antibiotics.

OP-11

Cytotoxic Compounds from Fagonia cretica L. Exerts p53 Dependent and Independent Apoptosis via DNA Damage in Human Breast Cancer Cell Lines

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Breast cancer is the most frequently diagnosed malignant disease in women. One out of every nine women in Pakistan faces risk of the disease due to which every year 40,000 women die in Pakistan. Fagonia cretica L. is a popular folk medicine and it is used for the treatment of breast cancer by local healers in Pakistan. Recently, for the first time we have isolated some selective cytotoxic compounds Quinovic acid, Quinovic acid-3β-O-β-D-glycopyranoside, Quinovic acid-3β-O-β-D-glucopyranosyl-(281)-β-D-glucopyranosyl ester and 12-(4-methyl-pent-3enoyloxy)-20-(4-methyl-pent-3enoyloxy)-3β,12β,20β-trihydroxy-pregnan-3-ylO-β-D-cymapyranosyl-(1→4)-3-methoxy-6-deoxy-β-D-glucopyranoside from ethyl acetate fraction of this plant. Using flow cytometric analysis of the cells, it was demonstrated that compounds exert time and dose dependent arrest of cell cycle at G0/G1 phase and apoptosis in MCF-7 and MDA-MB-231 human breast cancer cell lines with a reduced effect in normal human breast cell line MCF-10A.Expression of p53 protein and its downstream transcription targets, p21 and BAX were analyzed by western blotting, which revealed a p53 associated growth arrest within three hours and apoptosis within 24 hours of treatment with compound 1 and 4 in wild type MCF-7 cells. DNA double stranded breaks were measured as $^{\gamma}$-H2AX expression, which was detected early in both MCF-7 and MDA-MB-231 cells. Compounds 1, 2, 3 and 4also exert p53 independent growth arrest in MDA-MB-231 cells mainly by DNA damage response as represented by FOXO3a expression. Our results indicate that F.cretica L. is an important medicinal plant which contains potential anti-proliferative compounds against breast cancer cell lines which induce apoptosis via p53and FOXO3a expression.

OP-12

Larvicidal and Insecticidal Activity of Streptomyces Isolates of Salt Range, Pakistan

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A total of fifty-one different actinomycetes strains were isolated by selective isolation on glycerol casein KNO3 agar and actinomycete isolation agar (AIA). The isolates were screened for the production of insecticidal and larvicidal compounds against 3rd instar larvae of mosquitoes Culex quinquefasciatus and Tribolium castaneum (Red flour bettle). The biological screening revealed that the metabolites produced by these isolates have strong inhibitory effects on the growth of insect larvae, as the metabolites of three isolates exhibited 100% mortality of the tested larvae. In chemical screening by thin layer chromatography (TLC) and HPLC-UV, the crude extracts obtained from the culture broths of these saline actinomycetes isolates, exhibited an impressive diversity of the chemical constituents. The selected strains were identified based on their morphological, biochemical, physiological and cultural characteristics and by 16S rRNA gene sequence analysis. Among all the strains the isolates SA-9K, SA-9L and SA-10BC were detected as the potent producers of insecticidal compounds, these isolates exhibited maximum genetic similarity with Streptomyces rochei (99%), Streptomyces moderatus (92%) and Streptomyces sp. (98%) respectively. This study reports the larvicidal and insecticidal potential of the actinomycetes flora of salt range Pakistan.

OP-13

ATP Binding Cassette (ABC) Transporters as Drug Target

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ATP-binding cassette (ABC) transporters are the largest family of transmembrane proteins, which are named on basis of amino acid sequence identity in the ATP binding domains. Several of them play a vital physiological role in preventing xenotoxic substances from entering the body, thereby shielding it from potentially harmful substances. Among these multidrug resistance ABC transporters may play a major role in absorption, distribution, metabolism and elimination (ADME) of drugs. These transporters, in particular ABCB1 (P-gp) have been found associated with resistance of tumors towards anticancer drugs. It has arisen from a homodimeric ancestor by gene duplication. Crystal-structures of mouse MDR1A indicate that P-gp shares the overall architecture with two homodimeric bacterial exporters, Sav1866 and MsbA, which have complete rotational symmetry. For ABC-transporters nucleotide binding occurs in two symmetric positions in the motor domains. Based on the homology with entirely symmetric half-transporters the present study addressed the key question: Can biochemical evidence for the existence of dual drug translocation pathways in the transmembrane domains of P-gp be found? P-gp was photolabeled with propafenone analogs and labeling was assigned to two regions in the

protein by projecting data into homology models. Subsequently, symmetric residue pairs in the putative translocation pathways were identified and replaced by site directed mutagenesis. Transport assays corroborated the existence of two pseudosymmetric translocation pathways. While rhodamine123 has a preference to take one path, verapamil, propafenones and vinblastine preferentially use the other. Two major findings ensued from this study: the existence of two solute translocation pathways in P-gp and selective but not exclusive use of one of these pathways by different P-gp solutes. The pseudosymmetric behavior suggests an alternative concept of drug transport by P-gp that will aid in understanding the off-target quantitative structure activity relationships of P-gp interacting drugs.

OP-14

Targeting Drug Delivery of Anticancer Drug Conjugated with Magnetic Nanoparticles through Transferin Receptors

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Cancer has become the increasing cause of death all over the world. Efforts are being done for new methods and medicines to combat that deadly disease. Due to the various side effects of anti-cancer medicines on normal cells, targeting drug delivery in cancer cells has gained importance during the past few years. The expression of transferin receptors is increased on cancer cells due to increase metabolic rate and high uptake of iron particles. The transferin receptor can be used for selective targeting drug delivery. The magnetic nanoparticles (MNPs) are considered as a good nanovehicle for immobilization of anti-cancer medicines for targeting drug delivery. Transferin was conjugated with MNPs by glutaraldehyde activation method and then further conjugation with anti-cancer medicine (Epirubicin). The binding was confirmed by MagLISA. Affinity purified anti-transferin and anti-Epirubicin antibodies were used to confirm the coupling of transferin and Epirubicin. Results indicated that the binding of transferin and Epirubicin was found 59% to 62% and 45% respectively. HeLa and B-cells were cultured with MNPs-Transf-Epi conjugates separately and MagLISA indicated that the binding was 33% to 35% with HeLa cells and 12% to 15% with B cells. Results also indicated that the release of medicines at pH 5.0 is more (36% to 38%) than at pH 7.4 (11%). More killing effect of conjugates (15.2%) was observed on HeLa cells than B cells. Immunofluorescence and immunoperoxidase microscopy further confirmed the more expression of transferin receptors on HeLa cells than B-cells. The HeLa and B cells incubated with anti- HeLa and anti-B cells antibodies respectively were devoid of binding of conjugates. The dual property MNP can be used for binding of medicines and for targeting drug delivery.

Poster Presentations

PP-01

Co-Infection of Water Buffaloes in Punjab, Pakistan with *Neospora caninum* and *Brucella abortus*

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This study was aimed at ascertaining concomitant infection of *Neospora caninum* and *Brucella abortus* in dairy buffaloes in two districts (Lahore & Narowal) of Punjab province, Pakistan. For this purpose, 312 serum samples were randomly collected from the Nili Ravi breed of buffaloes during August 2009 to July 2010. A monoclonal antibody-based competitive Enzyme Linked Immunosorbent Assay (cELISA) was employed to test all the serum samples for the detection of *N. caninum* specific antibodies. The sera were further screened for *B. abortus* antibodies using the Rose Bengal plate agglutination test. The overall seroprevalence of *N. caninum* and *B. abortus* antibodies was 43.3% (95% CI ±3.4) and 12.2% (95% CI ±3.6), respectively. Largely, adult buffaloes (>3-7 years of age) exhibited higher prevalence of neosporosis and brucellosis compared to young buffaloes. Concomitant infection was quite considerable as 13.2% (95% CI ±5.1) of *N. caninum* infected (43.3%) buffaloes were also infected with *B. abortus*, suggesting relatively high abortion risk in such animals infected with both pathogens concurrently, than buffaloes infected with either one of the pathogen at a time. Adoption of efficient preventive measures as using effective vaccine, proper disposal of aborted fetuses and placental membranes, screening for infection and subsequent culling of infected animals could help to impede the spread of these abortifacient pathogens in Pakistan.

PP-02

Genetic Diversity and Structure Analysis of Wild and Land Race Barleys in Jordan Revealed by ISJ Markers

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The collections of crop germplasm resources are essential for on going plant breeding efforts. To exploit valuable genetic reservoirs, however, scientists need a vast knowledge about the extent and distribution of genetic diversity resided within collections. Current study was aimed to investigate the genetic diversity and genetic structure within and

between cultivated and wild barley populations in primary gene pool of barley at country scale in Jordan. A total of 7 populations comprising 4 barley landraces and 3 wild barleys were analyzed using 7 ISJ markers. The genetic diversity index He of barley landraces ranged from 0.049 to 0.060; while that of wild barley populations was ranged from 0.084 to 0.146, which suggests that wild gene pool of barley harbor significantly higher genetic diversity than its domesticated counterpart, a reflection that domestication of barley was accompanied by a genetic bottleneck. AMOVA revealed high genetic variations among populations rather than within the populations, indicating that high genetic differentiation of populations resulted by geographical and genetic isolation of the populations in the harsh environment of Middle East. PCA, clustering and STRUCTURE analysis distinguished not only wild and cultivated barley, but also each single population, representing their hereditary basis and original sample site. Our results also showed that there is lower genetic communication between wild and cultivated barley under natural conditions. The present findings put forward a range of implications and ramifications to the theories and practice for plant germplasm collection, conservation, and storage.

PP-03

Desert Actinomycetes: Isolation, Identification and Screening for Antimicrobial Potential against Methicilin Resistant Staphylocossus aureus (MRSA)

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The changing pattern of diseases and the emergence of resistant bacterial strains against currently used antibiotics continuously put demand for novel antibiotics. Actinomycetes is the most important group of bacteria for the production of medically valuable secondary metabolites specially antibiotics. The aim of this study was to screen the desert actinomycetes against methicillin resistant *Staphylococcus aureus* (MRSA). Thirty one strains of actinomycetes were isolated from the soil and sand samples of Cholistan desert. These isolated strains were characterized morphologically, biochemically, physiologically and genetically. Twenty five isolates exhibited promising antimicrobial activity against MRSA, however the isolate $S_7$19 was found to be the strongest inhibitor of almost all the tested MRSA strains while screened biologically in an agar diffusion assay. In chemical screening, the crude extracts were analyzed by thin layer chromatography (TLC) using different spraying reagents and by HPLC-UV/IR, the metabolic fingerprints of each of the extract demonstrated an impressive chemical diversity of bioactive secondary metabolites. The activity against MRSA and production of diverse metabolites reveal that the desert actinomycetes are the proliferant producers of useful antimicrobial agents, and should be screened further with respect to the novel drug discovery.

PP-04

Specialized Roles of the Conserved Subunit OST3/6 of the Oligosaccharyl Transferase Complex in Innate Immunity and Tolerance to Abiotic Stresses

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Asparagine linked glycosylation of proteins is an essential co- and post-translational protein modification in plants. The central step in this process is the transfer of a preassembled oligosaccharide to nascent proteins in the endoplasmic reticulum (ER) by the oligosaccharyltransferase (OST) complex. Despite the importance of the catalyzed reaction, the composition and the function of individual OST subunits are still ill-defined in plants. Here, we report the function of the highly conserved OST subunit OST3/6. We have identified a mutant in the OST3/6 gene that causes overall underglycosylation of proteins and affects the biogenesis of the receptor kinase EFR involved in innate immunity and the endo-β1,4-glucanase KORRIGAN1 required for cellulose biosynthesis. Notably, the ost3/6 mutation does not affect mutant variants of the receptor kinase BRI1. OST3/6 deficiency results in activation of the unfolded protein response and causes hypersensitivity to salt/osmotic stress and to the glycosylation inhibitor tunicamycin. Consistent with its role in protein glycosylation, OST3/6 resides in the ER and interacts with other subunits of the OST complex. Together, our findings reveal the importance of Arabidopsis thaliana OST3/6 for the efficient glycosylation of specific glycoproteins involved in different cellular processes and shed light on the composition and function of the plant OST complex.

PP-05

Identification of Potential Drug Targets against Streptococcus gordonii

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Streptococcus gordonii causes bacterial endocarditis by entering the blood stream usually after oral trauma. The complete genome of this bacteria and the host Homo sapiens was subjected to subtractive genome analysis with the objective of identifying potential drug targets. Innovative techniques are required to identify the drug target that has vital importance in curing various infections and diseases. Subtractive genomics is widely used in this process. Using this approach, in recent years, a large number of targets have been identified for bacterial pathogens that are either drug resistant or for which no suitable

vaccine is available. In silico method reduces the time as well as the cost of target screening. In subtractive genome analysis, homologous proteins of S. gordonii were excluded to prevent any chance of similarity with the host. The non-homologues proteins were analysed for sequence homology with the Database of Essential Genes to determine the essentiality of the proteins for the bacteria. These essential proteins were further analysed to predict the metabolic pathways in which they were involved. Furthermore, identification of subcellular localization of respecting metabolic pathways and functional characterization of essential genes were performed. From the complete genome of S. gordonii, 93 proteins were identified as potential drug targets, in which 13 proteins are involved from unique metabolic pathways. Drug prioritization of 93 proteins affirmed that the drug targets would be useful in design and discovery of novel therapeutic compounds against S. gordonii.

PP-06

Genetic Basis of Cadmium Induced Mood Disorder

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Mood is a persistent emotional state as differentiated from affect, which is the external display of feelings. Mood disorders “are a class of disorders marked by emotional disturbances that may spill over to disrupt physical, perceptual, social and thought processes”. Mood disorder is classified into three major groups that are Depressive disorders, Bipolar disorders and Substance induced mood disorders. Bipolar disorder is a mood disorder formerly known as "manic depression" and described by alternating periods of mania and depression. Bipolar disorder is a complex genetic disorder in which the core feature is pathological disturbance in mood (affect) ranging from extreme elation, or mania, to severe depression usually accompanied by disturbances in thinking and behavior.. In the studies, researchers found that there were six SNPs on chromosome 3p21 that could be associated with the presence of a major mood disorder. At the most significant marker, rs2251219, the presence of a C allele was consistently less common in individuals with major mood disorders than in those without. Genetic variations on Chromosome 3 were significantly associated with both mood disorders. The suspect gene, called PBRM1, codes for a protein critical for chromatin remodeling, a key process in regulating gene expression. Emerging evidence suggests individual genes do not cause depression, but they are thought to increase the probability of an individual having a depression in the face of other accumulating risk factors, such as other genes and environmental stressors.One gene that has been shown to increase the risk for depression in the context of multiple stressful life events is the gene for the serotonin transporter protein. Basic science experiments have shown that another gene, called BDNF, regulates the expression of a protein that is important for the ability of the serotonin gene to cause

these developmental effects. The BNDF gene plays a critical role in allowing the serotonin gene to have its affect on brain developmentchemical synaptic transmission in brain and peripheral and central nervous system affect by heavy metals including mercury, lead, cadmium, aluminum and others. These metals have adverse and severe effect on these systems. Cadmium may replace zinc in a number of ion channels, metalloenzymes and proteins due to metabolic compatibility of zinc and cadmium. Concentrations of noradrenalin and dopamine generally increases due to exposure of cadmium, it also impairs enzymes that are involved in the synthesis of neurotransmitters. Depending on age, brain region and duration of metal exposure serotonin production may be altered. Thus cadmium is potential neurotoxin, but metallothonein in the brain provided some level of protection against it. Cadmium has the ability to replace zinc in many critical enzyme systems and play main role in induction of mood disorder. Imbalance in the amount of neurotransmitters serotonin and noradrenaline cause mood disorder.

PP-07

Breast Cancer: An Overview from Pakistani Population

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Breast cancer is a leading cause of female mortality and morbidity worldwide. Data on the incidence and prevalence of breast cancer in Pakistan have historically been limited. The first report of cancer incidence data on the basis of the Karachi Cancer Registry was published in 2000, showing that it was the most common cancer in women. The incidence of breast cancer in Karachi was reported as the highest in all of Asia. A 2008 report by the Pink Ribbon Campaign Pakistan stated that 90,000 new breast cancer cases are detected annually, and the disease caused 40,000 deaths a year. It is likely that reproductive history, environmental and genetic factors all play a role. Pakistan has the highest rate of breast cancer in Asia and spends the lowest percentage of its gross domestic product on health. Dietary factors, obesity, use of oral contraceptives, age and family history are considered important in etiology of breast cancer. Infertility, old age, early menarche, late menopause and positive family history had been found to have relationship with occurrence of breast cancer in Pakistani females. An insight into the above data emphasizes the formulation of a BRCA1 and BRCA2 database for the Pakistani population. The high prevalence of these cancers and the presence of recurrent mutations of these genes in the Pakistani population, especially the observation of a high percentage of BRCA1 variants in ovarian cancer cases, emphasize the need for improving genetic counseling strategies and make genetic testing a part of screening policies. The more work done on the genomics of this disease with relevance to the Pakistani population, the closer a genetic cure targeted for this specific population can be found,. Most cases of breast cancer presented in advanced stage

probably due to poor economic status, illiteracy and negligence by patients or their family members.

PP-08

The Role of MTHFR; A→T Polymorphism as a Risk factor for Coronary Heart Diseases (CHD) and Hyperhomocysteinemia (HHC) in Chronic Hepatitis C Patients

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Hepatitis C virus is one of the major health problems in Pakistan. It affects mainly the liver but several extrahepatic manifestations were also accounted. Chronic hepatitis C patients are at an increased risk of developing hepatic steatosis, which share many clinical features with the metabolic syndrome. Hepatic steatosis has also been associated with elevated levels of markers of inflammation such as homocysteine, identified as hyperhomocysteinemia (HHC). HHC due to Methylenetetrahydrofolate Reductase (MTHFR) gene, in particular the C677T (Ala222Val) polymorphism were recently associated to CHD in chronic hepatitis C (CHC) patients. Homocysteine is an intermediate in methionine metabolism, which takes place mainly in the liver. Impaired liver function leads to altered methionine and homocysteine metabolism. The aim of this study is to evaluate the role of MTHFR; A→T polymorphism as a risk factor for Hyperhomocysteinemia (HHC) in chronic Hepatitis C patients and to determine its relationship with CHD in Pakistani population. For this study 5ml venous blood sample from patients and age and sex matched control individuals were collected randomly with informed consents from a primary health care hospital. Subjects were segregated into three groups: Healthy volunteers constituted the control group (Group I), HCV patients with CHD (Group II) and Chronic HCV (Group III). The homocysteine (Hcy) levels were determined by chemiluminescence method. DNA was isolated from the collected blood samples. MTHFR_C677T polymorphism, have been evaluated by PCR-RFLP analysis. The results were statistically evaluated by SPSS ver. 16. Our results indicate that plasma Hcy levels are highly prevalent in subjects with chronic hepatits C. The presence of genotype TT of MTHFR C677T polymorphism were significant in CHC in Pakistan. Moreover, using a physical parameter index, clinical characteristics and medical tests, we evaluated the role of genetic susceptibility of MTHFR C677T polymorphism with HHC and the extent of coronary disease in CHC patients. These results will help in understanding the association of both hyperhomocysteinemia and CHD in chronic HCV patients which may generate a lethal feedback loop each promoting the development of the other. Treatment of HHC might be promising to slow down the progression of CHD in hepatitis C patients.

PP-09

Dengue Infection in twin Cities (Rawalpindi/Islamabad): An Epidemiological Study

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Dengue virus is a single stranded positive sense RNA virus that belongs to the family Flaviviridae. Dengue virus exists as four serotypes, DENV-1 to 4 and is transmitted to humans via bite of Aedes (the mosquito). Infection with dengue is asymptomatic in the majority of cases, but it may also cause dengue fever, a debilitating flu-like illness that lasts for up to two weeks. In rare cases, infection results in dengue hemorrhagic fever or dengue shock syndrome, severe life threatening diseases characterized by high fever with vascular leakage and hemorrhage. The incidence of dengue has risen considerably over the recent decade and it is now a major public health problem. We included 183 patients with the mean age between 5 years to 48 years both male and female from April 2009 October 2010. The study was conducted in Islamabad diagnostic center. The blood was collected and the serum was obtained by centrifuging. The infection was diagnosed by detecting the IgM by ELISA DRG® Dengue Virus IgM (EIA-3471). Total of 183 patients including 62 (33.88%) were females with and 121 (66.12%) were males. The infected females were 13 (20.97%) while borderline cases were 02 (3.22%) and negative females were 47 (75.80%). The infected males were 41 (33.88%) while the borderline cases were 06 (4.95%) and negative males were 74 (61.15 %). From the results we concluded that the incidence of infection is higher in male than female. It leads to hypothesis that the male are more prone to get dengue infection than female.

PP-10

HCL: Hepato/Cardial Protective Plants Library

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For many decades, drugs have been used successful in the treatment of most diseases. Moreover, from ancient times to modern history, medicines based on traditional plants are playing a major role in health care. In Medicinal plant, phytochemicals are natural bioactive sources found in them acting as a defense to fight against several diseases. A number of bioactive compounds commonly obtained are proven to reduce cardiovascular and liver disease risks and helping in cardioprotection and hepatoprotection which is the leading cause of death globally. HCL database provides information regarding medicinal plant to

cure liver and heart diseases with reported targets, mechanisms, phytochemicals, and structures with original literature references. This system allows researchers to download one or complete phytochemical structures available in library in .mol and .pdb format.

PP-11

Antibacterial Activity of *Nigella sativa* (Kalvanji) Against Different Strains of *Salmonella* Characterized by Microsatellite Fingerprinting and 16s rRNA Sequencing

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Salmonella is a food borne pathogen and causative agent of human Salmonellosis and Typhoid fever causing many deaths in developing countries. Unnecessary use of antibiotics have stimulated the bacterial resistance which has adverse effects associated with the significantly increase in the occurrence of infectious diseases. Therefore, it is a need to develop alternative antibacterial drugs from medicinal plants and other natural extracts for the treatment of infectious diseases. The present study was planned to investigate the antibacterial effect of *Nigella sativa* (kalvanji) against different strains of *Salmonella*. Sixty bacterial strains were isolated and purified from different environmental sources including drinking water, waste water, sewerage water, Poultry, beef, eggs, fruits, vegetables and clinical samples. After Biochemical analysis, twelve bacterial strains were confirmed as *Salmonella*. Antibiotic susceptibility test was done by well diffusion assay against different concentrations of Ceftriaxone and Ciprofloxacin. The behaviour of both antibiotics was different against *Salmonella* strains. Six *Salmonella* strains resistant to both antibiotics were analysed for the antibacterial activity of natural extract of *Nigella sativa* (Kalvanji). Partitioning of Kalvanji powder was performed with Ethyle acetate. *Nigella sativa* oil extract was found to be more effective against *Salmonella* species for which even Ceftriaxone and Ciprofloxacin were ineffective. The genetic diversity of the selected *Salmonella* strains were analysed by microsatellite fingerprinting. Further molecular characterization of the selected strains was done by 16s rRNA sequencing.

PP-12

Association Mapping for Fertility in Trait in Dairy Cattle Bulls through High Density SNP Genotyping Array

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Reproductive efficiency is a most important determinant of dairy profitability. Fertility in the herd is absolutely critical for both male and female animals. Most of the molecular

genetics studies related to fertility in dairy cattle were directed toward the female side and very little importance has been placed on genetic aspects of fertility parameters of bulls. In the present study association mapping was carried out for fertility trait in Holstein dairy cattle bulls using high throughput high-density SNP genotyping array. Single nucleotide polymorphisms were associated with dairy cattle bull fertility traits. Associated SNPs were queried in the bovine genome. Seven SNPs were found within the genes and fourteen were within 10 kb of a gene. Seven genes, namely LEPREL1, MOBKL3, CD247, LRRC8A, LRFN5, ITIH1 and ENTPD1 were selected as candidate genes. Resequencing and fine mapping of these candidate genes were performed and identified SNPs were associated with dairy cattle bull fertility.

PP-13

Prevalence of Vitamin D Deficiency in Islamabad, Pakistan

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Vitamin D is one of the fat-soluble secosteriods that plays a vital role in the absorption of calcium and phosphorus from the intestines. Dietary intake and exposure to sun light are the major sources of vitamin D. It helps the bones to function efficiently while its deficiency leads to osteomalacia, rickets and could alter many metabolic pathways in several terms. Inadequate vitamin D is frequent and deficiencies can be found in all countries in all ethnic groups, and across all ages. In Pakistan, the frequency of vitamin D deficiency is not identified. This particular study was designed to determine the prevalence of vitamin D deficiency in general population of Islamabad and its suburbs. The study was conducted from January 2012 to December 2012 at the Department of Pathology, Kulsum International Hospital, Islamabad. During the study period, 737 subjects were tested for vitamin D concentration by using Electro-chemiluminescence Immuno Assay (ECLIA) technology. Out of a total of 737 subjects tested, females made up 76.2% of the sample. The mean age of respondents was 36.3 years (age range 15-75 years) years. 562 (76.2%) were females while 175 (23.8%) were males with a age range of 15-75 years. Females had significantly lower mean Vitamin D levels (56.2%) compared to males (15.3%). Insufficiency has been reported by 11.3% individuals with 9.65% females and 1.65% males. The rest of the subjects (17.2%) were normal including 10.5% females and 6.7% males. There are many factors which contribute to the vitamin D deficiency worldwide. These factors include reduced exposure to sunlight, age-linked reduction in cutaneous synthesis, and intake of food with a reduced vitamin D level. Our study has reported a high percentage of vitamin D deficient individuals and the frequency of vitamin D deficiency increased considerably with age and was greater in women. The highest prevalence of

vitamin D deficiency in females could be because females are not exposed to sunlight properly and are mostly house-wives involved in domestic work.

PP-14

Horizon in Therapeutics: Antimicrobial Action of Dendrobium nobile and Phalaenopsis Against Pyogenic Skin Infection Isolates

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Orchids are well-known around the globe as plants of decoration and are known as gems in the area of horticulture. The basic idea of this research study was the assessment of in vitro antimicrobial activity of the flower extracts of Dendrobium nobile and Phalaenopsis plants against Staphylococuus aureus and Staphylococcus epidermidis by means of agar disc diffusion method. Strains were isolated from skin acne patients and were identified by conventional methods. The flower aqueous and chloroform extracts of Dendrobium nobile and Phalaenopsis showed antibacterial activity against pyogenic skin isolates. In comparable, several antibiotics were tested alongside the isolated organisms. The information demonstrates potential outcome for Dendrobium nobile and Phalaenopsis in contrast to five different antibiotics. Moreover, analysis also confirmed that the pyogenic organisms were challenging besides several antibiotics. This study opens a new dimension whereby ornamental plant extracts may be employed for antimicrobial treatment.

PP-15

Cloning, Expression and Purification of Hypervariable Region of Hexon Gene of Fowl Adenovirus-4

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Hydropericardium syndrome (HPS) is an important re-emerging disease of poultry caused by Fowl adenovirus-4 (FAV-4). Present study was designed for expression and purification of recombinant hexon protein. An isolate of FAV-4 recovered from HPS in broiler breeder flocks was propagated in chicken embryo liver cell culture and hypervariable region of hexon gene was amplified by PCR using gene specific primers. Hexon was cloned into pTZ57R/T (TA cloning vector) sequenced and sub cloned into expression vector pGEX4T-1. Recombinant clones were analyzed by colony PCR and sequencing. Positive clones after

induction were analyzed for protein expression by SDS-PAGE. Expressed fusion protein was purified and confirmed by western blotting. Further studies to check the immunogenicity of recombinant hexon protein for development of subunit vaccine are in progress.

PP-16

Identification of Yellow Rust (Yr) Genes in Synthetic Hexaploid of Wheat

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In this study six SSR/STS markers were used for the identification of yr genes present in stripe rust resistant synthetic hexaploid of wheat. The plant material consisted of 51 stripe rust resistant synthetic hexaploid wheat accessions. A total of 68 alleles were detected through six gene linked markers. The mean number of allele was 11.3 alleles per locus. The largest number of alleles was associated with B genome (40 alleles) as compare to A genome (28 alleles). Genetic diversity values ranged from 0.34 to 0.93, with highest genetic diversity value of 0.93 detected for marker *xwmc-477*. The lowest genetic diversity value was observed for marker *xbarc-167.* The mean genetic diversity values were observed to be 0.56. In this study the PIC value ranged from 0.33 to 0.92 with an average of 0.55. Highest number of alleles were detected for marker *wmc-477* i.e. 24 alleles. This study showed the presence of *Yr15* in D67.2/P66.270//Ae. Squarrosa (497), D67.2/P66.270//Ae. Squarrosa (308) and SORA/Ae. Squarrosa (192). *YrR61* in CETA/*Aegilops Squarrosa* (392), GAN/*Ae. Squarrosa* (335), CETA/*Ae. Squarrosa* (417), SORA/*Ae. Squarrosa* (192), CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. Squarrosa* (1018), DVERD-2/ *Ae. Squarrosa* (1027) and CETA/*Ae. Squarrosa* (1027). *Yr5* SORA/ *Ae. Squarrosa* (192), ALTAR 84/ *Ae. Squarrosa* (192), DOY1/ *Ae. Squarrosa* (511) and SCA/ *Ae. Squarrosa* (518). *Yr48* in SORA/ *Ae. Squarrosa* (192), 68.111/RGB-U//WARD RESEL/3/STIL/4/ *Ae. Squarrosa* (385), DOY1/ *Ae. Squarrosa* (534), ALTAR 84/ *Ae. Squarrosa* (192), ALTAR 84/ *Ae. Squarrosa* (198), GARZA/BOY// *Ae. Squarrosa* (311) and YAR/ *Ae. Squarrosa* (783). *YrTP1* in GAN/ *Ae. Squarrosa* (335), CETA/ *Ae. Squarrosa* (417), SKARV_2/ *Ae. Squarrosa* (304), D67.2/P66.270// *Ae. Squarrosa* (497), D67.2/P66.270// *Ae. Squarrosa* (308), SORA/ *Ae. Squarrosa* (192), CETA/ *Ae. Squarrosa* (1038), DOY1/ *Ae. Squarrosa* (534), ALTAR 84/ *Ae. Squarrosa* (192), ALTAR 84/ *Ae. Squarrosa* (198) and 68.111/RGB-U//WARD/3/FG/4 RAB/5/ *Ae. Squarrosa* (882). *Yr 36* DOY1/ *Ae. Squarrosa* (534), DOY1/ *Ae. Squarrosa* (447) and 68.111/RGB-U//WARD/3/ *Ae. Squarrosa* (511). One the basis of *Yr* linked SSR marker analysis most genetically diverse lines are D67.2/P66.270// *Ae. Squarrosa* (497), CETA/ *Ae. Squarrosa* (386), CETA/ *Ae. Squarrosa* (1031), CPI/GEDIZ/3/GOO//JO/CRA/4/ *Ae. Squarrosa* (1018), DVERD_2/ *Ae. Squarrosa* (214), ALTAR 84/ *Ae. Squarrosa* (502), SCA/

Ae. Squarrosa (409), STY-US/CETA//PALS/3/SRN_5/4/ *Ae. Squarrosa* (502), CETA/ *Ae. Squarrosa* (1024), 68.111/RGB-U//WARD/3/ *Ae. Squarrosa* (511).

PP-17

Bioremediation of Mercury Compounds by using Immobilized Nitrogen-fixing Bacteria

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The mercury contamination is of great concern because of its toxicity and ubiquity. The environmental level of mercury is rising day by day because of anthropogenic activities. Bearing in mind the toxicity of mercury, in this study, we have isolated various strains of nitrogen-fixing bacteria (NFB) from nodules of different plants on YEM medium. The preliminary screening of NFB strains to resist mercury was done by well plate assay. Characterization of the selected strains for the production of hydrogen sulfide (H_2S) was done by growing them on LA medium. The hydrogen sulfide producing NFB strains co-precipitated the mercury in the form of HgS resulting in transformation from toxic (Hg^{2+}) to non-toxic (Hg^0) form. Further screening was done by quantitative assay by using Dithizone method. Mercury resistant and hydrogen sulfide producing NFB strains, characterized by biochemical tests as *Enterobacter, Cronobacter and Pseudomonas*, gave the most promising results in the detoxification of mercury. Finally these strains were immobilized in sodium alginate (synthetic beads) and their ability to detoxify mercury containing industrial effluents was determined as compared to free cells by Dithizone method. It was concluded that immobilized NFB bacteria detoxified more concentration of mercury as compared to free cell cultures.

PP-18

Overexpression of *EXPANSIN* Maintains the Integrity of Cell Wall and Shows Tolerance to the Negative Effect of Growth Inhibitors; A Potential Target for Crop Improvement in Agriculture

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Mechanical properties of plant tissues are determined by the cell wall that surrounds the cell. Expansins are cell wall proteins associated with cell wall loosening during different stages of plant development. Here in this paper we study the effect of *EXPANSIN A8* on the growth of plant in transgenic *Arabidopsis thaliana* by generating overexpression plants. The coding sequence of *AtExpA8* was expressed under the control of the CaMV 35S

promoter in individual*Arabidopsis thaliana* plants. Transgenic lines of the T3 generation were tested by isolating total RNA and subsequent cDNA synthesis using oligo-dT18 primers and mRNAs as templates. The expression of the target gene was analyzed through quantitative real-time polymerase chain reaction (qRT-PCR) to confirm increased level. These lines were then grown on a growth inhibiting drug and compared with the control. The *AtExpA8* overexpression lines showed high tolerance to the negative effects of the drug. Furthermore, these lines were tolerant to high concentration of sucrose and salt supplemented in the growth medium. This could be used for improvement of crops to tolerate different kind of stresses pose to agriculture in Pakistan.

PP-19

Bacterial Auxin: Comparative Study of Growth Induction in *Arabidopsis thaliana L.* and *Triticum aestivum L.*

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Rhizobacteria belonging to Bacillus, Pseudomonas, Micrococcus, Escherichia and Staphylococcus genera were evaluated to elucidate the role of bacterial auxin signaling in enhancing the growth of Arabidopsis thaliana (L.) and Triticum aestivum L. Strains were confirmed for auxin biosynthesis by colorimetric and GC-TOFMS (gas chromatography and time-of-flight mass spectrometry) analysis. In this study, A. thaliana L. wild type Columbia (Col-0) in comparison with mutant lines defective in hormone response pathways; aux1-7 (insensitive to auxin and ethylene), axr4-1 (insensitive to auxin) and eir1-1 (insensitive to ethylene) were used as a model system. Bacterization of wild type Col-0 recorded significant increases in shoot length (38%) and number of siliqua (180%) with Bacillus subtilis TpP-1, over water treated control. Inoculation of aux1-7 and axr4-1 showed lack of growth response or statistically comparable results for different growth parameters as compared to their respective control. Treatment of eir1-1 with Pseudomonas sp. AvH-4 and B. subtilis TpP-1 recorded significant increases for shoot length (9%) and number of siliqua (20%), respectively. The pattern of growth response of aux1-7, axr4-1 and eir1-1 indicated that bacterial auxin and ethylene signaling is involved in the growth promotion of A. thaliana. After bacteria-Arabidopsis experiments, bacterization of T. aestivum seeds also recorded significant increases for shoot length (29%), number of tillers (74%) and seed weight (26%) with Pseudomonas sp. AvH-4. Results of this study suggested that the growth induction by B. subtilis TpP-1, Pseudomonas sp. AvH-4 and P. aeruginosa As-17 in A. thaliana is associated with bacterial auxin signaling. Moreover, comparison of growth response of A. thaliana and T. aestivum demonstrated that auxin producing rhizobacteria can be used to inoculate cereal crops to enhance their productivity.

PP-20

Transgenic *Artemisia dubia* WALL Showed Altered Phytochemistry and Pharmacology

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In order to test the effect of trans-genes (*rol* ABC) on possible high production of biologically important phytochemicals and enhanced pharmacological activities, three transgenic lines (1, 2 and 3) of *Artemisia dubia* WALL (transformed with *Agrobacterium tumefacienes* harboring *rol* ABC genes) were subjected to phytochemical analysis and pharmacological studies. Earlier this plant was selected for transformation experiments due to its high pharmaceutical value. A great variation in phytochemistry and the pharmacological activities was observed not only between the transgenic and non-transgenic control plants but also among the transgenic lines itself. Comparative chemical profile obtained via HPLC, TLC and Spectrophotometer showed high degree of variations in the quantity of phytochemicals, like increased production of total flavonoids (71.1% in transgenic line 2) and total phenolics (110.8% in transgenic line 1), increase in caffeic acid and catechin and a decrease in gallic acid in the extracts of transformed plants compared to the untransformed controlled plants. In case of pharmacological activities moderate to high level increase in antimicrobial (antibacterial and antifungal) activities, cytotoxicity (14.1%), antitumor (29%) and antioxidant activities (23.9%) was observed (in transgenic line 2). Although all the three transgenic lines under study had shown an increase, however, overall assessment of pharmacological activities was such that transgenic line 2>1>3>control.

PP-21

Physiological, Biochemical and Molecular Changes Associated With Chromium Exposure in Maize

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Chromium(Cr)contamination of soil can be effectively remediated by crops having high potential of phytoextraction.Maize (Zea mays L.) is a widely grownstaple cereal with promising attributes of a heavy metal accumulator. Understanding the response of a plant to a stress requires a comprehensive evaluation of stress-induced changes at various physiological biochemical and molecular levels.The potential of phytoextraction of maize was assessed on the basis ofchlorophyll content, lipid peroxidation, proline content,Cr

accumulation in root and shoot, hormonal changes of various plant growth regulators, differential expression of proteins and metabolite profiling.Plants were subjected to different concentrations of chromium supplied through soil and associated changes of various attributes of maize were measured accordingly.

PP-22

Controlled Assembly of Plasmonic Nanoparticles via Biological Spacers for Molecular Sensing

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The electric field enhancement between two interlinked plasmonic nanoparticles is subject of intensive research nowadays. When brought in close proximity, the plasmons of individual nanoparticles can couple resulting in enhanced optical properties. Such properties can be utilized in applications such as SERS for biological sensing such as DNA sequencing and single molecule detection. In the present study we investigated, the effect of dimerization of nanoparticles that can be utilized as active SERS components Amphiphilic polymer coated gold nanoparticles are active platforms for immobilization of various molecules bearing NH2-functionality. Biotin functionalized gold nanoparticles, core diameter 4 nm, have been fabricated by utilizing the carboxylic group on the nanoparticle surface and amino functionality of Biotinylated Polyethylene glycol (MW 5000 Dalton). The two components i.e. Biotin-tagged and streptavidin tagged nanoparticles were commingled to get the dimeric assemblies of colloidal nanoparticles. The final yield of the reaction was 33%, as confirmed by transmission electron microscopy (TEM) images.

PP-23

Association of Single Nucleotide Polymorphism in KCNQ1 and ADIPO Q Gene with Type 2 Diabetes Mellitus in Pakistan

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The proportion of type 2 diabetes in Asia is increasing and it shows no signs of slowing. With this fastest growth, type 2 diabetes disorder is being seen in this population. Several genes play important role in causing this disorder. KCNQ1 and ADIPOQ are among one of important genes that encodes for voltage-gated potassium channel and adiponectin protein respectively. Researchers have investigated KCNQ1 expression in insulin secreting cells

and it might have a role in impaired pancreatic β cell function therefore mutation in the gene has been associated with type 2 diabetes. Likewise adiponectin produced in adipose tissue has been involved in number of metabolic processes like glucose regulation and in the suppression of metabolic derangements that may result in type 2 diabetes mellitus. Researchers have investigated single nucleotide polymorphism in KCNQ1 (rs2237895) and Adipo Q (rs2241766) has been associated with type 2 diabetes mellitus. This study incorporated 155 T2DM patients and 56 control patients. Their blood samples were collected and were processed for DNA extraction through phenol chloroform method than PCR-RFLP technique is used for genotyping of allelic variants rs2237895 and rs2241766. Bioinformatics analysis has been done by different softwares. Statistical evaluation was conducted by SPSS software. The purpose of this study is to investigate association of single nucleotide polymorphism rs2237895 (A/C), in KCNQ1 gene and rs2241766 (T/G) in Adipo Q with type 2 diabetes in Pakistani population. We did not find any significant association of either of the SNP with type 2 diabetes mellitus patients. Our results have revealed the non-significant correlation of single nucleotide polymorphism rs2237895 in KCNQ1 gene and rs2241766 in Adipo Q gene with type 2 diabetes mellitus in Pakistani population.

PP-24

New Cost-Effective In-vitro Propagation of Ornamental Plants and Evaluation of Antimicrobial Activities against Staphylococci

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Tissue culturing is extensively used as a successful technique for the propagation of numerous plants. This venture is focused on the propagation of ornamental plants by using simple and low estimated media not including any additional adjuvant. Ixora coccinea, Draceana Reflexa (Song of India), Alternanthera (Purple Knight), Kiwi (Actinidia deliciosa) and Cestrum Nocturnum (Raat Ki Rani) were subjected for the initiation. Murashige and Skoog media were used in the present study supplemented with Benzylamniopurine (BAP) 1.5mg/l and Kinetin (KIN) 1.5mg/L. Among five plants, three plants successfully showed the results while two of them did not show positive results. Draceana, Alternanthera, Ixora successfully nurture in the lab condition while Kiwi and Cestrum Nocturnum were unsuccessful to grow in the medium. The successfully propagated ornamental plants (Ixora coccinea, Draceana Reflexa (Song of India), Alternanthera (Purple Knight) were then tested for the antimicrobial activity. The leaves aqueous and chloroform extracts showed potential antibacterial activity against Staphylococcus aureus and Staphylococcus epidermidis. Seven different antibiotic discs were also tested in comparison to the ornamental plants. Such a cocktail may reduce the threat of rapid resistance development against several antibiotics.

PP-25

Antimicrobial Potential of Some Important Jordanian Medicinal Plants

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Most of the health problems are due to different infectious diseases caused by different microorganisms. Since herbal medicines can be a cure of these diseases thus scientists have developed interest to discover the hidden treasures of herbal medicines. The present study has been conducted to check the antimicrobial activity of crude protein extract of some Jordanian medicinal plant seeds by disc diffusion method. These selected plants include *Triticum dicoccoides, Hordeum spontaneum, Phagnalon rupestre, Hedypnois rhagadioloides, Asphodeline lutea, Artedia squamata, Mandragora autumnalis, Alcea setosa.* Among these plants, *Triticum dicoccoides* showed maximum zone of inhibition against fungal strain *Alternaria alternata*. This study leads to the synthesis of antibiotics after isolation and purification of antimicrobial compounds.

PP-26

Antimicrobial Activity and Minimum Inhibitory Concentration of Ethanolic Leaf Extract of Punica Granatum

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In the present study, we evaluated the antimicrobial activity and minimum inhibitory concentration in the ethanolic leaf extract of Punica granatum against pathogenic bacteria like Pasteurella multocida, Bacillus subtilis (BGSC 10419), Streptococcus aureus, Methicillin-sensitive Streptococcus aureus and Methicillin-resistant Streptococcus aureus. The ethanolic extract of the leaves was found to possess strong antimicrobial activity against Pasteurella multocida, Bacillus subtilis (BGSC 10419), Streptococcus aureus as revealed by in vitro agar well diffusion method. The minimum inhibitory concentration of extract was ranged between 50-150 mg/ml. Plant extract was also investigated for the presence of different phytochemicals by performing preliminary phytochemical tests and TLC.

PP-27

Genetic Basis of Cadmium Induced Autism

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Autism is a genetic disorder, is a lifelong condition that affects the way in which a person communicates and relates to the people around them and also affects the way in which the world is perceived and thus how they make sense of it. It is a spectrum condition which is characterized by severe and pervasive impairments in several important areas of development: reciprocal social interaction and communication as well as behavior and imagination. Research from 2009 suggests autism now affects every 1 in 110 children. And that number appears to be only increasing. After diagnosis many factors are obtained that causes autism and one in Cadmium. Molecular studies reveal that ninety nine percent of autistic children in a recent study have dysfunctional metallothionein metabolism. Metallothionein is a protein that binds to toxic cadmium and allows the body to eliminate the cadmium and other toxic metals. If this binding protein doesn't function well, one is not able to transport or eliminate these metals. A number of factors which regulate the gene expression include microRNAs, and the level of microRNAs differs between postmortem autism brains and control brains. The single case study was carried out which revealed the supports involved in educating and socializing school-aged children with autism spectrum disorder. The case study indicated that the parents agreed on a majority of issues and clearly pointed out that their major area of concern was education. Treatments involve many therapies but the major is parenting tips to guide parents in the upbringing of their autistic Childs.

PP-28

Association of XRCC1 (194, 280 and 399) and XPD (751) Polymorphisms with Acute Coronary Artery Disease

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Acute Coronary Syndrome (ACS) is the most common disease and cause of mortality across the world and certain risk factors i.e. age, gender, smoking, diabetes, hypertension, drugs usage, weight etc are known to be associated with the disease. The aim of this study was to find if there is any correlation between ACS and hereditary genetic defects in DNA repair gene, X-ray cross-complementing group 1 (XRCC1), which is involve in base excision repair (BER) in DNA. Three single nucleotide polymorphisms (SNPs) [Arg 194 Trp, Gln Arg 280 His and Arg 399 Gln] of XRCC1 and one SNP from XPD (Lys 751Gln) were determined in 221 subjects, from Southern Punjab (in Pakistan) population, comprising (115 ACS patients and 106 healthy control) by PCR–RFLP and genotype at both codon were individually as well as in combined form was correlated with the risk factors associated with ACS. Our results indicated that all four genotypes Arg 194 Trp ($p = 0.903$), Arg 280 His ($p = 0.851$), Arg 399 Gln ($p= 0.228$) and Lys 751 Gln ($p= 0.953$) were not associated with ACS either individually or in combined form ($P=0.718$). When various studied parameters were compared between patients suffering from various forms of ACS and their healthy controls,

it was observed that age (P = 0.05), gender (P < 0.001), education (P < 0.001), family history (P = 0.005), hypertension (P < 0.001), diabetes (P < 0.001) and smoking habit (P = 0.002) were the significantly different parameters among them and may be associated with the incidence of cardiovascular diseases.

PP-29

DNA Integrity and Morphology of Human Male Gamete is Compromised after Mild Increase in Testicular and Epididymal Temperature

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Objective: To investigate, for the first time in men, the effects of a mild increase (2°C) in testicular and epidiyaml temparature on sperm chromatin integrity Design and Methodology: Testicular and epidiymal hyperthermia was created by diurnal cryptorchidism method. Testes of fertile volunteers were lifted up and maintained at the inguinal position for 15h daily for 120 consecutive days. Semen samples were collected before, during and after hyperthermia according to the chronology of human spermatogenesis. Sperm count, sperm motility and morphology were assessed by routine laboratory method. Sperm DNA fragmentation index (DFI) and high DNA stainability (HDS) were analysed by sperm chromatin structure assay (SCSA). Results: Mean (±SEM) sperm DFI (%) was significantly increased ($p<0.05$) compared to control (11.9±1.5) at day 20 (D20) (16.7±3.9), D34 (23.8±2.9) and D45 (31.3±5.4) during hyperthermia and remained higher during the entire hyperthermia period till D45 after hyperthermia. Sperm HDS (%) started to increase as early as D20 (7.4±1.5) during hyperthermia and was significantly higher than control (5.9±0.3) at D34 (10.9±1.0) and D45 (13.0±1.1) respectively and remained higher during the entire hyperthermia period until D45 after hyperthermia. Index of multiple anomalies of sperm morphology was significantly higher at D9 during hyperthermia. Percentage of motile spermatozoa decreased as early as D20 and total sperm count decreased as early as D34 during hyperthermia and remained decreased until D45 after hyperthermia. Sperm DFI/HDS, total sperm count and percentage of motile sperm returned to their respective control values at D73 after cessation of hyperthermia. Conclusions: Our results implicate that sperm DNA damage occurs before any drop in sperm count even with a mild increase in testicular-epididymal temperature and is a pre indicator of alterations in sperm characteristics. Therefore, subsequent potential interest involves male contraception specifically during inhibition and recovery phases of spermatogenesis, male infertility and assisted reproductive technology.

PP-30

Phosphate Solubilizing Bacteria are Effective and Economical Biocontrol Agents

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Phosphorus (P) is one of the foremost plant growth-limiting nutrients although it is plentiful in soils in both inorganic and organic forms. Phosphate solubilizing micro-organisms (PSMs) are ever-present in soils and could play significant title role in bio control activity against pathogenic fungi providing eco-friendly and defensible mode. Biological control of soil borne plant pathogens is a potential substitute to the use of chemical pesticides which are unsafe to the environment. In the present study phosphate solubilizing bacteria were isolated from rhizosphere soil of different plants growing in different region of Lahore and Okara Districts of Punjab (Pakistan) and tested for the biocontrol activity against soil borne pathogenic fungi like Sclerotium rolfsii, Fusarium oxysporum, Macrophomina phasiolina and Alternaria alternate under invitro condtions. Six phosphate solubilizing bacteria were identified including Citrobacter freundii, Klebsiella pneumonia, Enterobacter aerogenes, Burkholederia cepacia, Acinetobacter lwoffi and Proteous vulgaris and all the six bacterial strains were found as effective biocontrol agents against various soil borne plant pathogenic fungi. The growth of Macrophomina phasiolina was significantly controlled by Citrobacter freundii, Klebsiella pneumonia, Enterobacter aerogenes, Burkholederia cepacia; and of Acinetobacter lwoffi. Alternaria alternate was controlled by Citrobacter freundii, Klebsiella pneumonia, Enterobacter aerogenes and Burkholederia cepacia. The growth of Fusarium oxysporum was controlled by Citrobacter freundii, Klebsiella pneumonia, Enterobacter aerogenes, Burkholederia cepacia where as that of Sclerotium rolfsii was negatively affected by Citrobacter freundii, Klebsiella pneumonia, abd Enterobacter aerogenes. The result of the present study encourages the need to explore more PSMs that can improve our economy indirectly by exerting beneficial influence on crop production.

PP-31

Antioxidant Treated Young and Aging Mesenchymal Stem Cells: Role in Regenerative Medicine

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Cell-based interventions have been implicated in treatment for a broad spectrum of diseases and are being explored to provide reparative. In cell-based regenerative medicine, stem cells are being widely investigated due to their ability of tissue regeneration, capacity of self-renewal and stemness, and the property of plasticity and differentiation. Though, ideal for cell-based treatment, use of stem cells for different diseases is impeded by several factors including loss of cells on transplantation due to the cell death on encountering the ischemic environment of the impaired tissue or organ. The deteriorating regeneration potential is associated with advancing age. With advancing age, stem cells also age and this is accompanied by adult stem cell functional changes, which is thought to be the cause behind the aberrant guidance of cellular differentiation in cell-based therapy. Moreover, stem cells also go through life-long exposure to several extrinsic threats with advancing age including reactive oxygen species (ROS), biological toxins, physical stressors, hazardous chemicals, etc. These insults can accelerate the processes of cellular senescence. Present study explores the molecular mechanisms associated with the aging of MSCs by comparing these in young and aged MSCs. The study is also aimed at evaluating the effects of antioxidants on the aging of MSCs. In this study, MSCs from young (yMSCs) and aging (oMSCs) rats were used and were compared on the basis of morphological changes and the doubling time of the cultured cells. The present study evaluates the cell survival, quantification of oxidative stress, effects of the antioxidants and transcriptional profiling of several genes involved in various pathways in both the groups under hypoxia and oxidative stress. The study demonstrates that mesenchymal stem cells (MSCs) particularly oMSCs can be preconditioned with antioxidants thus improving the regenerative potential of these cells for cell-based therapeutics for various diseases.

PP-32

Establishment of In-vitro Regeneration and Genetic Transformation of Groundnut (Arachis hypogaea L) Varieties under Various Hormonal Regimes

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Legumes are the most important group of crop plants next to cereals and much effort has been devoted to develop efficient in vitro regeneration systems because of their recalcitrance to tissue culture regeneration. There is also not much success with genetic transformation of Groundnut (Arachis sp.) have been achieved due to the lack of efficient protocols to obtain whole plants through in vitro regeneration of adventitious shoot buds from the transformed tissues. Most of the legumes are recalcitrant to Genetic Transformation. This study was conducted to optimize the media for establishment of efficient regeneration protocol of three Groundnut varieties which was GOLDEN, BARI2000 and Bard479 for Genetic Transformation. There were different concentration and combination of hormones like NAA, BAP. IBA used. Embryo slices were used as an explants

tested on Murashige and Skoog (MS) basal salt medium supplemented with B5 vitamin. The optimum concentration of 6 benzylaminopurine (BAP) for shoot regeneration was 4mg/l with an α Naphthalene acetic acid (NAA) 0.1mg/l. while for rooting Indole Butyric Acid (IBA) concentration was 1mg/l confirmed. BAP was found more suitable for shooting regeneration with NAA. Whole plants were regenerated from in vitro cultured sectioned of embryo slices. The genetic transformation of herbicide resistant gene (EPSPS) was transformed successfully in Groundnut. The Agrobacterium strain LBA4404 with a plasmid V-ZMGT32 harboring the desired gene for herbicide resistant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS).

PP-33

Molecular Characteerization of MDR-TB in Pakistan

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Mycobacterium tuberculosis remains one of the most significant causes of death from an infectious agent, leading to 2 million deaths annually worldwide (Doustdar et al., 2008). The incidence of tuberculosis in Pakistan is 181 per 100,000 individuals, but information about transmission and geographical prevalence of Mycobacterium tuberculosis strains, their evolutionary genetics and drug resistance remains limited (Tanveer et al., 2008). The major problem faced in the control of this infection Worldwide is the emergence of multidrug resistance tuberculosis (MDR TB), typified by simultaneous resistance of M. tuberculosis to both isoniazid and rifampicin (Dye et al., 1999). Multi-drug resistant tuberculosis (MDR TB) is reported both in developed and developing countries and is a public health problem especially in the developing world. Many biological and socioeconomic factors like sub-optimal chemotherapy are responsible for the emergence of drug-resistant TB (Long, 2000). To date, 11 genes have been linked to resistance to the first line anti-TB drugs: katG, inhA ,inhA promoter, ahpC, kasA and ndh for INH resistance (Victor et al., 2002); rpoB for RIF resistance embB for EMB resistance, pncA for pyrazinimide (PZA) resistance and rpsL and rrs for streptomycin (STR) resistance (Wade et al., 2004). 200 MDR-TB samples have been collected from different regions of Pakistan including Lahore, Multan, Karachi, Rawalpindi and Peshawar and they will be further investigated by 24 locus MIRU-VNTR technique.

PP-34

Expression of GLUT1 in Leukemic Cells

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Marked elevation in glucose uptake is one of the characteristic features of cancer cells and it is also a well-known clinical marker for the identification of these cells. At least four of the glycolysis related genes - GLUT1, PGI/AMF, G6PD and TKTL1 – are known to be involved in oncogenesis. Previous reports suggest that GLUT1 acts as an oncogene in multiple types of cancers. Heightened expression of GLUT1 has been observed in different types of malignancies such as hepatic, pancreatic, breast, esophageal, brain, renal, lung, cutaneous, colorectal, endometrial, ovarian and cervical carcinoma. GLUT1 is also a major glucose transporter in leukemia cells, and the modulation of glucose transport activity by cytokines, oncogenes or metabolic stresses is vital for their existence and proliferation Altered expression of various key regulatory genes plays a significant role in the development of leukemia. The molecular mechanism by which leukemic cells go on proliferating along with their altered metabolism is also related to GLUT1 mutations but has not been entirely revealed yet now, though it may include many other factors. The present aims to determine the role of GLUT1 in progression of hematopoietic cancers. We performed mutational analysis for the promoter region of GLUT1. The mutations in this region are known to affect activity and expression of GLUT1 protein. In addition to that we compared expression levels of GLUT1 protein between cancer patients and control subject.

PP-35

Characterization of some Dicot Promoters for their Exprression in *Nicotiana tabacum*

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Plant transformation is important genetic engineering tool for introducing foreign genes into plant genomes. The expression of transgenes is regulated by the promoter attached upstream to the gene. The different promoters from the same category (induced, constitutive or differential) may have different strengths for the expression of a gene. The major constrain regarding the utilization of commercially available promoters is the intellectual property right (IPR), therefore, discovery of efficient new promoters is important considering the IPR issues or the efficiency of expression. The theme of this research was focused on stable transformation, using GUS expression cassettes under selected promoter sequences, in Nicotiana tabacum via Agrobacterium-mediated transformation and perform expression analysis via histochemical GUS assay. Four promoter sequences from i) Aquaporin (AQP), ii) Sucrose Phosphate synthase (SPS), iii) Sucrose synthase (SUS) and iv) Beta tubulin (BT) were selected and cloned in plant expression vector (pGA482). The expression cassettes were constructed in a modified vector derived from pJIT166 (pGR1) that contains GUS with intron under 2X35S promoter followed by CaMV terminator. The four GUS expression cassettes were developed by replacing 2X 35S promoter in pGR1, with the four selected promoters. The four GUS

expression cassettes were verified through restriction analysis and PCR amplifications. The tobacco transgenics for each expression cassettes were obtained. PCR positive transgenic plants were then stained for GUS expression to check the expression levels for the selected promoters. The results are presented along with the proposed utilization of the studied promoters.

PP-36

Screening of Biosurfactant Producing Bacteria and their Role in Oil Biodegradation

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Biosurfactants are surface active compounds produced by the microorganisms. They have potential to solubilise hydrocarbon contaminants and make them available for microbial degradation. In the present study, Biosurfactant producing bacterial strains isolated from oil contaminated soil and oil from drilling wells and screened them for biosurfactant production by using different screening tests. All bacterial strains were further characterized morphologically (colony morphology, gram staining, spore staining, capsule staining and motility test), biochemically (Catalase, oxidase, Urease, Nitrate reduction, Lactose fermentation, Oxidation fermentation, Methyl red, Citrate utilization, Indole test, Casein hydrolysis, Starch hydrolysis, Lipid hydrolysis, Gelatin hydrolysis etc.), physiologically (pH, Temperature, Antibiotic, heavy metals, antimicrobial activity) and genetically (plasmid isolation, Plasmid detection and 16S rRNA sequencing). Strains which showed positive drop collapse and stable emulsion formation were selected as positive strains for biosurfactant production. On the basis of 16SrRNA gene sequencing analysis strains S5-1 identified as Acinetobacter (100% homology) S1-8 as Enterobacter (99% homology) and S7-1 as Pseudomonas Stutzeri (100% homology).They showed resistance to different metals and antibioics and showed antimicrobial activities against pathogenic strains. On plasmid isolation clear bands of plasmids were observed in all strains.The complete decolorization of redox indicator 2, 6 dichlorophenol indophenol (DCPIP) at different concentrations of oil (1%, 1.5%, 2%, and 2.5%) was observed in case of S5-1, S1-8 and S7-1 which showed their ability to degrade oil. Biosurfactant producing strains have showed appreciable oil degrading ability which can be exploited for bioremediation of oil contaminated sites and can be helpful in oil spill removal in soil and aquifers which have very harmful effects on environment.

PP-37

Hydrogen Peroxide Induced Oxidative Stress and Response of Antioxidant Systems in *Camellia sinensis* Treated Lymphocytes

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Free radicals put in to more than one hundred disorders in humans. Environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress contribute in their production. They cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Recently there has been an upsurge of attention in the therapeutic potentials of medicinal plants. Numerous natural antioxidants have already been isolated from different varieties of plant material such as leaf vegetables, fruits, seeds, cereals and algae. Their protective mechanism can be explained by the capacity of the antioxidants compounds e.g. phenolics, flavonoids and polypropanoids in the plants and plant products themselves to scavenge free radicals, due to their proton donating ability. Research methodology of present study involved isolation and extraction of plant extracts, screening of phytochemical components, determination of antioxidant activity and its effect on living cells using lymphocytes as a model system. *Camellia sinensis* (green tea) extracts were prepared in three different solvents viz. aqua, methanol and ethanol. Initially, phytochemical screening involved determination of ascorbic acid, phenolic, flavonoids and flavonol contents. Antioxidant activity determination included scavenging assays e.g. DPPH assay and ABTS assay, HPLC, hydrogen peroxide scavenging assay and reducing power assay. *In-vitro* antioxidant actions were determined by the activities of catalase (ELISA kit method), superoxide dismutase, lipid peroxidation and total protein contents on lymphocyte cell lines. The present study revealed that *Camellia sinensis* has high contents of ascorbic acid, phenols, flavonoids, and flavonol. It was also a good scavenger of oxidants as evident by DPPH, ABTS and reducing power assay. *In-vitro* antioxidant results also showed positive effects in lymphocyte cell culturing. Results of extraction with solvent methanol showed the highest antioxidant activity. Experimental plant is potential source of natural antioxidants.

PP-38

Transcriptomics of Pseudomonas sp. Isolted from Different Water Regimes

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Present attempt is to evaluate the relative expression of dessication resistance genes in Pseudomonas strains isolated from rhizosphere of maize and rice grown at varying moisture regimes. The Pseudomonas sp. (1) and Pseudomonas sp. (3) were isolated from rhizosphere soil of maize grown in irrigated fields (soil moisture 18%) and semi- arid region (soil moisture 12%) respectively, while Pseudomonas sp. (2) and Pseudomonas sp. (4) were isolated from raised bed (soil moisture 16%) and irrigated fields (soil moisture 28%) of rice respectively. Viability of Pseudomonas cells under dessicated (10% RH) and hydrated (85% RH) conditions was observed by using plate counts analysis following incubation period of 4h at 4°C. Cell viability of all Pseudomonas sp. decreased significantly under dessicated (10% RH) condition as compared to that of hydrated (85% RH) conditions. All four Pseudomonas strains were positive for trehalose synthase (tre), Sigma factor (rpoS), 2,3 butanediol (adh), Acetion dehydrogenase (acoA), Alginate (algF), Flagellar protein (flgG), flagellar motor switch protein (fliM), Alginate regulatory protein (mucA) genes while Flagellar protein (flgG) was not detected in Pseudomonas sp.(4) isolated from irrigated fields of rice fields. RT-PCR showed that Pseudomonas sp. increase significantly relative expression of trehalose synthase (tre), Sigma factor (rpoS), Alginate regulatory gene (mucA) and Flagellar motor switch protein gene (fliM) in desiccated (10% RH) condition. Marked up-regulation in relative expression of trehalose synthase (tre), Sigma factor (rpoS), Alginate regulatory gene (mucA) and Flagellar motor switch protein (fliM) gene in response to induced desiccation (10% RH) of Pseudomonas sp. cells in relation to their water stressed habitat (from rhizosphere of rice grown in irrigated and raised bed condition) was observed. It is inferred from the study that trehalose synthase (tre), Sigma factor (rpoS), Alginate regulatory gene (mucA) and Flagellar motor switch protein gene (fliM) plays a prominent role in promoting desiccation tolerance in bacteria. The up-regulation of mucA genes in response to desiccation (10% RH) stress may induce exopolysaccharides production in the Pseudomonas strains isolated from water scarce environment (semi-arid and raised bed).

PP-39

Harvest and Culture of Plucked Human Hair Follicle Cells In-vitro

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Human hair follicles are miniature hair growing organs. Androgenetic alopecia (AGA) is common human hair disorder. AGA is progressive, patterned hair loss from the scalp *In vitro* culturing of the plucked hair follicle cells is the best method to apply the cultured cells back into the balding scalp. Plucked human hairs are cheap source to treat male baldness. Therefore, it may become possible to create thousands of hair follicles from that original follicle. The aims and objectives of the present study were to isolate human hair follicle cells of normal and AGA groups by plucking as it is the cheap source of human tissue; to

harvest the hair follicles cells of normal and AGA *in vitro* without feeder layer and to study the morphology of the cultured cells of plucked human hair follicle of both the groups. The plucked human hair follicles were taken from both the groups. The whole procedure of harvesting and culturing of plucked human hair follicle cells was done under extreme sterile conditions. The morphology of cultured and subcultured cells was observed under phase contrast microscope for 14 days. Keratinocytes appeared after 24 hours, Melanocytes appeared at 48 hours, and stem cells appeared in 7 to 10 days. Shelf life of cultured and subcultured cells of normal and AGA group was 12 and 7 days respectively. Live cell counting by using improved Neubauer chamber, DNA extraction and Optical Density (OD) assay was also done of cultured and subcultured cells of plucked human hair follicles of both the groups. The results of present study showed that the plucked human hair follicles cells were harvested and cultured successfully without feeder layer. Their genomic DNA was extracted successfully. Hence, this hair cloning technique is cost effective method as compared to the hair transplantation. The foremost advantage of the new technique compared to hair transplantation is the preservation of the 'donor hair area'.

PP-40

Late Enbryogenesis Abundance Protein gene HVA1 under a Stress Inductive Promoter RD29A, Improves Drought and Salinity Tolerance of Wheat *(Triticum aestivum L.)*

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Drought and salinity are two major abiotic factors involved in plant growth retardation and yield reduction of major crops worldwide. Millions of Acres of fertile farm land is laying baron is Pakistan due to these stresses. Wheat, staple food for millions of Pakistani people, also suffers yield losses due to drought and salinity. Late Embryogenesis Abundance (LEA) protein gene HVA1 plays major role in membrane protection, osmotic regulation, desiccation tolerance and cell membrane integrity during different abiotic stresses. In current study HVA1 gene under stress inductive promoter rd29A has been transformed in local wheat cultivar Seher-06 by employing Agrobacterium mediated transformation technique. Transgenic plants were confirmed for gene integration through PCR, GUS histochemical assay, southern hybridization, semi quantitative RT-PCR and Basta leaf bioassay. Finally five transgenic events were selected and tested under three drought (full, half and no irrigation) and two salinity (10 dSm-1 and 20 dSm-1) treatments. In case of drought stress at booting stage WUE, RWC and photosynthetic rate, event P76 surpasses all the transgenes including wild type whereas in case of Transpiration rate and stomatal conductance events P78 and PQ were on top, respectively. On 10 dSm-1 salt stress events P78 and P82-9 showed higher flag leaf area, plant height, spike length, dry root weight,

shoot weight, root length and grain yield in comparison to wild type. Whereas all the transgenic lines produced more proline and higher K+ accumulation under salinity stress as compared to control plants.

PP-41

Purification and Identification of Bioactive Angucyclinones from Streptomyces Matensis BG5, Isolated from the Rhizosphere of Roza indica L.

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A newly isolated strain Streptomyces sp. BG5 was investigated for the production of bioactive compounds. The strain exhibited broad spectrum activity against an array of nine test organisms including gram positive bacteria, gram-negative bacteria, fungal and microalgal pathogens, along with a moderate cytotoxic response (28.9% mortality) in a microwell cytotoxicity assay against the brine shrimp Artimia salina. The morphological, physiological, and biochemical characterization of the Streptomyces sp. BG5 strongly suggested it to be a member of the genus Streptomyces. The nucleotide sequence of 16S rRNA gene (1433 pb) of the Streptomyces sp. BG5 (Gene bank accession number EU301836) exhibited high similarity (98%) with Streptomyces matensis. The large-scale fermentation of Streptomyces sp. BG5 and subsequent extraction, isolation, and purification of the crude extract afforded three pure compounds. The structures of these compounds were identified as ochromycinone (1a), emycin D (2), and 1-acetyl-β-carbolin (3), based on nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, and by comparison with reference data from the literature.

PP-42

Induction of Antifungal Gene in Potato through Agrobacterium Mediated Transformation and Molecular Analysis

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Potato is an important cash crop of Pakistan cultivated over an area 172.7 thousand hectares with an annual production of 3767.2 thousand tones (GOP, 2012-13).Potato production in Pakistan is low as compare to other countries. There are several factors responsible for low yield of potato crop in Pakistan. Virus free plant production is only

possible through tissue culture techniques while disease resistance plant could be produced through genetic manipulation because of its tetraploid nature. Chitanse has the ability to hydrolyze the chitin a (N-acetyl glucosamine polymer that makes up fungal cell walls) component of cell wall of most of the fungi. Genetic transformation of potato was carried for the induction of Chitinase gene through Agrobacterium mediated transformation system into two potato verities. The transgenic plants were produced on selection media containing hygromycin @ 20 mg/l. Hygromycin resistance and chitinase gene was present in the plasmid vector the construct (pBI333-EN4-RCG3) was KINDLY provided by National Institute of Agriculture Resources (NIAR) Tskuba, Ibaraki Japan. Transformed plants were confirmed by PCR analysis which revealed the presence of chitinase and hygromycin. After confirmation the culture were multiplied in vitro. The expression analysis of chitinase gene was carried by RTPCR which has confirmed that chitinase gene is expressing in leaf and stem tissues of transgenic potato plants. Transgenic potato plants of two varieties were planted under containment facility of NIGAB. The survival percentage of the transgenic plants was 80%.Transgenic plants were tested against (Rhizictonia and Fusarium solani). All of the fungal pathogens were grown on PDA media and incubated at 25oC. The Pathogenesity tests of transgenic potato plants were carried out in collaboration with Crop Disease Research Institute and Integrate Pest Management NARC. The transgenic plants containing chitinase gene showed resistance against fungal pathogens (Rhizictonia and Fusarium solani).

PP-43

Antimicrobial Assay Based Screening of Some Northern Area Medicinal Plants of Pakistan

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Medicinal plants occupy distinct position in the treatment and prevention of several diseases since very long. The use of medicinal herbs reduces the chances of side effects which may occur by the use of synthetic medicines. A study was conducted to determine the antimicrobial potential of some selected Northern area medicinal plants of Pakistan including *Trigonella foenum-graecum, Sisymbrium irio, Cheiranthus cheiri, Cuscuta reflexa Roxb* and *Lespedeza cuneata*. Their seed extracts were tested against a range of bacterial and fungal strains by Disc Diffusion method. Positive and negative controls were used to check the absence or presence of zones of inhibition. Among the medicinal plants, maximum zone of inhibition was observed by *Cuscuta reflexa Roxb* up to 7.3 cm against *Fusarium solani*. The results obtained justifies that all of these medicinal plants possess significant antimicrobial potential that will lead to drug discovery.

PP-44

Phylogenetic Analysis of Halotolerant L-Glutaminase Producing Bacteria from Kalar Kahar and Their Impact on Plant Growth

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The study of the bacterial diversity of saline environments is substantial in order to determine the potentials of halotolerant bacterial ecology in the saline ecosystem. A total of 50 halotolerant bacterial strains were isolated from Kalar Kahar and were screened for L-glutaminase activity. Ten strains were found positive for L-glutaminase activity and were characterized morphologically, biochemically, physiologically and genetically. Bacterial isolates were identified by 16S rRNA gene sequencing. Obtained sequences were BLAST in NCBI, seven strains showed close homology with genus Bacillus, and three isolates showed close evolutionary relation with genus Pseudomonas. Plant-microbe interaction results showed that the percentage germination and number of leaves remained almost similar in case of all inoculated as well as un-inoculated plants. Inoculated halotolerant bacterial strains substantially increased the dry matter of the plant. Few strains caused some increment in shoot length while in majority of strains this effect was negligible. Decrease in the length of roots was also observed in case of three isolates. This study can be used as a model for the industrial production of L-glutaminase enzyme from halotolerant bacteria as well as their use in plant growth promotion.

PP-45

Inhibition of Sesame Seedling Growth by Xanthomonas campestris pv. sesami

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Xanthomonas campestris pv. sesami (Xcs) is rod shaped, gram negative bacterium. This bacterial pathogen causes the bacterial blight of sesame and significantly reduces its yield. Severely infected leaves defoliate and spots are formed on the twigs. Present study was conducted to check the effect of culture filtrate of Xcs on germination and seedling of sesame. Different concentrations of culture filtrates of Xcs in MS (Murashige and Skoog) medium were used to monitor its effect on seed germination, height and root length of sesame seedlings. It was observed that Xcs greatly affects the length of root and length of whole seedling and slows down the process of germination. Smallest root and seedling height was obtained with 4% culture filtrate while there was normal growth in control sesame seedlings.

PP-46

Mutational Analysis of p53 Gene in Sporadic Breast Carcinoma

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Breast cancer is the most common malignancy among females worldwide and more than 1,000,000 new cases are diagnosed every year. p53 accounts for 12-46% of sporadic carcinoma of breast. In this study all analyzed tumors were sporadic. The PCR-SSCP technique is economical, convenient, fast, safe and popular in clinical research for the mutational analysis. In the present study, mutational analysis of p53 gene in exon 5, 7, 8 and 9 has been done by Single Strand Conformational Polymorphism (SSCP). DNA was isolated from 30 tumor samples and after PCR followed by SSCP, ten samples showed mutations in exon 8 (mutation rate 33.3%), eight mutations were observed in exon 7 (mutation rate 26.6%) and mutation in one sample was found in exon 5 (mutation rate 3.3%) in these 30 tumors. While exon 9 shows no mutation. For exon 8, four samples showing additional band, mobility shift and band deletion as compared to control in SSCP were selected for sequencing. Out of these four samples, two showed polymorphism when compared with the sequencing pattern of the control, one showed deletion of C after codon 281 and other showed insertion of T between codon 293 and 294. Overall, 19 different potential mutations had been detected by SSCP in present study showing 63.3% mutation rate and two mutations (6.66%) were confirmed by sequencing and proved this as a significant data. Suggested mutation of exon 5 and exon 7 by SSCP would be confirmed through DNA sequencing.

PP-47

Investigation of ZEB1/Delta-EF1 Gene Expression in Breast Cancer Tissue of Pakistani Women

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In Asian population Pakistan has the highest incidence rate of breast cancer which accounts for one third of all female cancers while it is second leading cause of cancer related deaths of females globally. ZEB1 gene has recently gained exclusive relevance in the field of molecular oncology for its role in tumorigenesis, tumor invasiveness and metastasis, and resistance to chemotherapy. Our study investigated ZEB1gene expression in ten breast cancer tissue and ten respective adjacent breast normal tissue specimens collected at the time of surgery and immediately snap frozen in liquid nitrogen. Total RNA was extracted trough trizol reagent and quality and quantity was estimated through NanoDrop1000 spectrophotometer giving A260/A280 ratio in the range of 1.8-2.2 signify the purity and

integrity of the total RNA which were processed to cDNA. The primers were designed for exon 6 and exon 8 of ZEB1 gene and the cDNA was amplified with those primers through polymerase chain reaction. We found that invasive ductal carcinoma constitute majority i.e. 80% of all breast cancer, while invasive lobular carcinoma account for significant minority i.e. 20% of all breast cancer cases. Expression of Zeb1 gene was observed in 90% cases of ductal carcinoma of breast and in 50% cases of lobular breast carcinoma while 20% cases of breast cancer showed no expression of Zeb1 gene. All of the breast normal tissue except one showed negative results for ZEB1 gene expression. The positive results were further confirmed by sequencing the PCR product. The sequences aligned exactly with the reference sequence. These results demonstrate that ZEB1 gene expressed in invasive breast tumor tissue with almost no expression in the normal tissue of the breast.

PP-48

Antimicrobial Activity of Secondary Metabolites from Fumaria parviflora Lam. (Fumariaceae)

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Antibacterial activity of the plant extracts and three compounds viz., nonacosane-10-ol (alcohol), 23a-homostigmast-5-en-3ß-ol (homolog of β-sterol) and cis- and trans-prtopinium (alkaloid) of Fumaria parviflora Lam. were in vitro assessed using well diffusion test, agar dilution method and minimum inhibitory concentration against seven clinical Gram (-) and Gram (+) bacteria. The zone of inhibition (IZ) and the activity index (AI) were maximum for Gram (-) Escherichia coli (IZ = 28 ± 0.9; AI = 0.93 ± 0.3), Klebsiella pneumonia (IZ = 22 ± 0.4; AI = 0.73 ± 0.4) and Salmonella typhi (IZ = 22 ± 0.4; AI = 0.73 ± 0.9) and these bacteria were strongly sensitive (SS) to the n-hexane root extracts. The minimum inhibitory concentration value for the plant extracts was found to be in the range of 3.12 to 50.0 mg ml-1. The three compounds displayed strong antibacterial activity at a conc. of 100, 200 and 300 µg ml-1 against the tested strains. The zone of inhibition of these compounds ranged from minimum (9 ± 0.9, AI = 0.3 ± 0.5) for Salmonella typhimurium to mximum (46 ± 0.9, AI = 1.53 ± 0.3) for E. coli. The cis- and trans-protopinum was the most potent antibacterial compound against all the strains tested at the highest conc. of 300 µg ml-1. The three compounds were completely bactericidal as measured by the viable cell count studies. Fumaria parviflora derived extracts and the phytochemicals possess antibiotic properties and these compounds could be used in the development of novel chemotherapuric agents.

PP-49

Diversity in Local Common Buckwheat Genotypes from Karakorum Mountains of Pakistan based on SDS-PAGE

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Amongst the currently existing plant resources of the world, unconventional crops like buckwheat posses significance due to its short life cycle and having high nutritive values and the best source of high biological value proteins among the plant kingdom in addition to have excellent supplementary value to the cereal grains. Thus present study was performed to evaluate the potential characters of Common buckwheat (F.esculentum Moench) genotypes based on SDS-PAGE from two different districts of Gilgit-Baltistan. Qualitative characters based on seed morphology and seed protein profiles of 12 local genotypes were evaluated for diversity assessment and cluster analysis. The seed proteins were resolved on 12.25% SDS-PAGE. The Cluster analysis based on Euclidean distance coefficients and the unweighted pair-group method with arithmetic mean (UPGMA) by STATISTICA revealed two lineages (L 1 and L 2) at linkage distance 0.39 and divided buckwheat genotypes into 5 main groups at 0.19 (50%) linkage distances. The genotypes from Yuchung, Askoli, Siksa and Baltoro showed 100% similarity, as compared to other genotypes. The highest percent disagreement was observed for Stak genotype (56 %) against genotype from Sadpara and Sino each, whereas genotype Stak also showed 50 % disagreement against Dambudas. Maximum of the protein bands on SDS PAGE were scored in the range of 55 KDa to 26 KDa. The protein profiling exhibited moderate level of genetic diversity among 12 genotypes. The findings would be more useful to evaluate agronomic characters as well as a base for varietal development programs in mountain communities for sustainable agriculture. The present study is the first attempt to study diversity in protein banding patterns among indigenous common buckwheat genotypes based on SDS-PAGE from Karakorum Range, Gilgit-Baltistan.

PP-50

Association of a Novel Mutation of MyoG Gene with Meat Quality Traits of Chinese Indegeneous Cattle Breeds

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The candidate gene approach allows identification of SNPs in genes, which likely to cause variation in a phenotypic trait based on physiological and endocrine evidence. The candidate genes underlying within a QTL region are positional candidate genes and association of these genes with production traits provides an excellent opportunity for marker assisted selection. Myogenin (MyoG) gene has mapped at 25 to 73 cm interval on BTA 16 where several quantitative trait loci for meat quantity and meat quality (marbling) are located. Present research was aimed to determine the associations between gene-specific single nucleotide polymorphisms (SNP) in MyoG gene, to investigate whether this polymorphism affected meat quality characteristics and to evaluate the allelic and genotypic frequencies of six native Chinese cattle breeds. The breeds were Jiaxian red (JXR), Luxi (LX), Nan-yang (NY), Qinchuan (QC), Xia-Nan (XN) and Xue long (XL). Results of our research suggested a transition of A → G at position 959 in exon 1 of the MyoG gene in cattle that caused the substitution (959Serine/959Cysteine). The A959G SNP was significantly associated with water holding capacity and meat tenderness ($P < 0.05$), while no effect of genotype on back fat thickness, rib area, loin eye height, eye muscle width and marbling was disclosed ($P > 0.05$). The x2-test revealed that the genotype distributions among the five cattle breeds (JXR, LX, NY, QC and XL) agreed with Hardy-Weinberg equilibrium ($P > 0.05$), although, one breed (XN) was not in Hardy-Weinberg equilibrium ($P < 0.01$). We concluded that, A959G SNP can be used as an efficacious genetic marker for meat quality traits in native Chinese cattle breeds but a much large number of animals are required for Marker assisted selection.

PP-51

Case Control GWAS (Genome Wide Genetic Association Studies) in Cardiovascular Diseases

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GWAS refers to a case control genetic study design based on a binary categorical variable in which individuals are usually classified as either affected or unaffected, attempts to identify, measure and analyze commonly occurring novel genetic sequence variants from across the human DNA genome that contribute to disease risk in the population of a large number of subjects without bias towards any particular candidate gene. This analysis method has a huge impact on the field of human genetics and has forced the genetics community to think on a genome-wide scale since the beginning of the 21st century. GWAS uses genetic risk factors affecting susceptibility to common diseases prominently cardiovascular disease (CVD) and identifies the biological basis of genetic disease susceptibility for developing improved preventive measures and new treatment strategies in the field of pharmacogenetics. Integrating different levels of complex cardiovascular

biomedical data along with their coupling with experimental systems is the future of human molecular genetics.

PP-52

Role of Natriuretic Peptides, Interleukins, Renin Angiotensin Aldosterone System and Estrogen in Cardiac Hypertrophy

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The mechanisms linking hypertrophy to renin-angiotensin-aldosterone system have not been fully established, but, Ang-(1-7)/Mas ablation (Ang-(1-7)/Mas-/-) and ovariectomy (OVX) may reflect impaired natriuretic peptides and interleukins levels. Four groups of female FVB/N mice; wild type females (WF); Ang-(1-7)/Mas-/- females (KF); ovariectomized (OVX) females (WFO); and Ang-(1-7)/Mas-/- OVX females (KFO) were used in RT-PCR, and immunohistochemical (IHC) analyses to relate morphological and histological findings. Histological and morphological analyses of hearts depict LV hypertrophy in KF and WFO in concurrence with significantly decreased natriuretic peptides expression reflecting a significantly high interleukins expression levels. It is here speculated that Ang-(1-7)/Mas-/- and OVX effect distinct mechanism of synthesis and secretion of natriuretic peptides as well as interleukins in hypertrophied female mice.

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PP-53

Prevalence of Vitamin D Deficiency in Local Adult Females of Pakistan

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Many epidemiological studies in recent years suggest that vitamin D is associated with a high risk of osteoporotic fractures, malignancy of breast, colon, prostate and many respiratory diseases. As sun is a rich source of vitamin D so once it was thought that vitamin D deficiency is rare in Asia. But many studies in India proved vitamin D deficiency in healthy subjects. Then later some researchers in Pakistan also reported vitamin D deficiency in some regions of Pakistan. This study was aimed to investigate the prevalence

of vitamin D deficiency in adult female of local population in Pakistan and to find out social and genetic factors that may contribute in hypovitaminosis. For this purpose blood samples from the local population has been collected, before sample collection a detailed interview was done to ask about their life style particularly about sun exposure. Renal and liver function test was done for screening any pseudo cause of hypovitaminosis. Serum Calcium and Phosphorus was done as a biomarker of vitamin D deficiency. Serum 25 hydroxy vitamin D estimation was done by EIA method. It has been observed that in adult female of Pakistan most of them have vitamin D insufficiency and some of them were deficient while serum calcium is not a significant biomarker to check the level of vitamin D. In obese female vitamin D insufficiency is more than non obese. Life style is a more significant factor that contributes in vitamin D insufficiency even in a sun rich country like Pakistan. Subjects that had vitamin D deficiency not show any significant clinical sign of any disease so this situation of vitamin D deficiency could be ignore at early stage that may lead to some drastic health effects in later stages that may be other then bone disorders. Further we are working on VDR genotyping of these subjects.

PP-54

Monitoring Biochemical Changes during Grape Berry Development in Portugese Cultivars by NMR Spectroscopy

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1H nuclear magnetic resonance (NMR) was applied for the metabolic profiling of grapes from three Portuguese cultivars including 'Trincadeira', 'Aragonês', and 'Touriga Nacional', at four developmental stages. Two kinds of extraction methods including deuterated NMR solvent extraction and solid phase extraction (SPE) were used for the metabolomic analysis and all the metabolites detected in 1H NMR were elucidated by twodimensional NMR techniques as well as the in-house NMR chemical shift database. Multivariate data analyses were also performed to identify overall metabolic differences. Trincadeira was found different from the other two cultivars, having low phenolic contents as compared to other cultivars. The initial stages showed comparatively high phenolics and organic acid contents like caftaric and malic acid while the later stages showed higher glucose and fructose levels. Veraison was found to be a metabolically critical stage of berry development. On the basis of these findings distribution of metabolites among different cultivars at different developmental stages is discussed. These results were integrated with transcriptional

profiling obtained using genome array to provide new information regarding the network of events leading to grape ripening.

PP-55

Cloning and Characterization of Hordeum vulgare Vacuolar Na+/H+ Antiporter Gene for Salinity Tolerance.

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Salinity has adverse effects on plant growth and productivity. Salt tolerant plant evolved mechanism of Na+ exclusion from shoot. Salinity tolerance mechanism involved sequestration of Na+ ion from cytosol into vacuole. The sequestration of Na+ is mediated by vacuolar Na+/H+ antiporters. Overexpression of NHX1 gene is reported to enhance salt tolerance in plants so by increasing the activity of NHX1, salt tolerance in the plant can be enhanced. In the recent study, a full length HvNHX1 gene was cloned under three different promoters i.e. ZmUbi1, OsAct1 and 2x35S using gateway technology. Tobacco plants have been transformed using agrobacterium mediated transformation method and resulting transgenic plants are being investigated for successful integration of HvNHX1 gene for salt tolerance at 100mM 150mM and 200mM NaCl. Expressional analysis of putative transgenic plants will also be studied.

PP-56

Dextran Production by *Weissella cibaria* CMGDEX3 Isolated from Cabbage

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Dextran is a polysaccharide composed of D-glucose units and features substantial number (at least 50%) of consecutive α (1→6) glycosidic linkage in the main chain and α (1→2), α (1→3) or α (1→4) branch glycosidic linkages. Due to the potential of dextran for commercial, nutritional and health applications, it is widely used in chemical, food and pharmaceutical industries. Several lactic acid bacteria are well known to utilize sucrose as a specific substrate to produce dextran. Recently *Weissella* species have been reported to produce good quality dextran and have shown promising applications in several sectors particularly in sourdough baking and probiotics production. In the present study dextran producing *Weissella cibaria* CMGDEX3 was isolated from cabbage on sucrose containing De Man, Rogosa and Sharpe medium. The structural characterization of purified dextran determined by FTIR, 1H and 13C NMR spectroscopy demonstrated that *W. cibaria*

CMGDEX3 synthesized a linear dextran that predominately had α (1→6) glycosidic linkages with only a few (3.4%) α (1→3) linked branches. Molecular mass determination showed that it was a high molecular weight dextran of an average >2,000,000 Daltons. Higher molecular weight dextran with few branch linkages is considered a good quality dextran. High molecular weight linear dextran of *W. cibaria* CMGDEX3 can be significant in various industrial applications particularly in sourdough baking. This is the first report on isolation of dextran synthesizing Weissella genus from Pakistan

PP-57

Callus Induction and Plant regeneration in Three Local Cultivers of Indica Rice (*Oryza sativa L*)

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Improvement of rice production is mainly dependent on the suitable regeneration protocol for the locally preferred rice cultivars. In this study we tried to establish a process of rice callus induction in three local rice cultivars. Seeds of three rice varieties (super basmati, MG and 1121) were evaluated for in vitro callus induction using Murashige and Skoog medium (MS) medium supplemented with different concentrations (2mg/l, 2.5mg/l, 3mg/l and 3.5mg/l) of 2,4-Dichlorophenoxy acetic acid (2, 4-D). The influence of two carbon sources i.e. maltose and sucrose on callus induction at different concentrations (3%, 4% and 5%) were also investigated. Maximum callus induction was observed at 3mg/l concentration of 2, 4-D in all rice varieties. At 3mg/l concentration of 2, 4-D, the percentage of callus induction was maximum for super basmati (85%) followed by MG rice (82%) and 1121 rice (75%). Among different sugar sources, maltose was most effective, resulting in highest frequencies of embryogenic callus formation. At concentration of 4% maltose, 76% callus induction was observed.

PP-58

Isolation and Characterization of Lytic Bacteriophages against Pathogenic Bacteria

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The hospital acquired infections are becoming a major challenge for patient care due to emergence of resistance against commonly used antibiotics. Today, phage therapy can replace antibiotic treatment, which has been a cause of emergence and rapid spread of antibiotic resistance. Bacteriophages are the bacterial viruses that either can lyse (lytic phages) the bacterial cell or can integrate their genome in the bacterial genome (lysogenic

phages) during their life cycle. The lytic phages and their gene products can easily be used as therapeutic agents against bacteria as they are host specific and show no side effects. In the present research work, bacteriophages of different plaque morphologies were isolated against *Enterobacter cloacae, Klebsiella pneumonae* and *Pseudomonas aeruginosa* from the sewage water and hospital effluent samples. These bacteriophages showed lysis activity against the clinical isolates and exhibited narrow host range. The lytic activity of these bacteriophages at varying temperatures and pH establish their wide stability range while highest viability was observed at 37°C and pH 7.0. The *Enterobacter* phages TSE1, TSE2 and TSE3, the *Klebsiella* phages TSK1, TSK2 and TSK3, and the *Pseudomonas* phages JHP1 and JHP3 were viable at high temperatures till 60°C. While the *Klebsiella* phage TSK1 worked at an alkaline pH of 9.0. All the phages efficiently reduced bacterial growth in the bacterial reduction assay. The *Pseudomonas aeruginosa* phages predominently maintained their lytic phase of life cycle throughout the 24 hours. The Calcium ion majorly enhanced the adsorption rate of all the phages to their hosts except the *Enterobacter* phages TSE1, TSE2 and TSE3. The proper manipulation of these highly active phages, their extracted genome and protein analysis can be an ultimate key to their better application in phage therapy. As the initial low dose can eradicate the bacterial infection locally so, it signifies the underlying potential of the bacteriophage therapeutics.

PP-59

Field Evaluation and Biosafety Studies of Basmati Rice Expressing Two Bt Genes in Pakistan

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Insect pests are one of the major threats to rice crop. Two Bt genes, cry1Ac and cry2A, were transformed in basmati rice for resistance against target insects and evaluated in the field for resistance to Yellow Stem Borer (YSB) and Rice Leaf Folder (RLF) over several years. They were analyzed for insect resistance, morphological, physiochemical properties and biosafety studies. Transgenic lines were 96-100% and 98% resistant against YSB and RLF respectively. Transgenic plants showed morphological variations, e.g., average number of tillers, plant height and maturity. Biosafety studies were also carried out. Fates of Bt proteins in soil, effect of Bt protein on non-target insects, risks of vertical and horizontal gene flow were evaluated. Bt protein was unstable and degraded significantly in soil within 30 days after harvesting the crop. No harmful effects were found on non-target insects. Maximum gene flow of 0.02% was observed. No allelopathic effects were found on germination and growth of chickpea and wheat crops. Moreover, due to short in stature, transgenic lines were observed to be resistant to lodging. In conclusion, the transgenic rice

plants transformed with Bt genes have significant resistance against YSB and RLF while no harmful effects on the environment.

PP-60

Identification of Potential Drug targets of Staphylococcus aureus Through Computational Studies

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The prolonged use of antibiotics over the years has transformed many organisms resistant to multiple drugs. This has made the field of drug discovery of vital importance in curing various infections and diseases. Advances in complete genome sequencing, bioinformatics and cheminformatics represent an attractive approach to identify drug targets worthy of experimental follow-up. Subtractive genomics approach is one of the recently adopted methodology in which the subtraction of sequence between the host and parasite proteome provides information for a set of proteins that are likely to be essential to the parasite but absent in the host. *Staphylococcus aureus* is a gram positive bacterium that has developed resistance to drugs. It causes pneumonia, skin infections, respiratory disease (e.g. sinusitis), and food poisoning. The main objective of this study was to identify potential drug targets. The study revealed such proteins which are non-homologous to human genome. Screening these proteins using the Database of Essential Genes (DEG) resulted in the identification of essential proteins of the bacterium. Analysis of the identified essential proteins, using the KEGG Automated Annotation Server (KAAS) housed at Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways database, revealed crucial enzymes that may be used as potential drug targets. Comparative modeling, molecular docking, virtual screening and molecular dynamic simulation studies as a follow up will be value addition for drug designing purposes especially for the current study.

PP-61

Anomalies of TRAIL Pathway in MALT Lymphoma

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Purpose: We investigated the expression profiles of TNF-Related Apoptosis Inducing Ligand (TRAIL) pathway components and the mechanisms underlying TRAIL-induced apoptosis in MALT lymphoma. In lymphoid malignancies these have only been explored in

a few non-Hodgkin lymphomas and in multiple myeloma so far; therefore, we have performed a pilot study to assess the potential role of TRAIL pathway in MALT lymphoma with and without cytogenetic aberrations. Methods: In 32 patients diagnosed with MALT lymphoma, we assessed genetic aberrations including t(11;18)(q21;q21), t(14;18)(q32;q21) involving IGH and MALT1, t(1;14)(p22;q32), t(3;14)(q14;q32) involving FOXP1 and IGH and trisomies 3, 8 and/or 18 by RT-PCR and FISH. In addition, we also investigated paraffin embedded specimens for the expression of the key components of TRAIL pathway including TRAIL, death receptors (DR) 4 and 5, decoy receptors (DcR) 1 and 2, and FLICE inhibitory protein (FLIPL) by immunohistochemistry. Results: All of the patients under study showed some alterations in TRAIL pathway mainly involving loss of death receptors (12 patients, 37.5%), gain of decoy receptors (1 patient, 3.1%) or both (19 patients, 59.4%). DR4 was coexpressed with DcR1and DcR2 with the correlation coefficient values R=0.376 (p=0.040) and R=0.713 (p<0.001), respectively. DcR2 was highly expressed in the tissues with normal cytogenetic status as compared to the group with cytogenetic aberrations (p=0.005). Moreover, DcR2 expression also correlated significantly with NF-κB expression (R=0.372,p=0.047). Conclusion: TRAIL components' expression is altered in MALT lymphoma. High expression of DcR2 in patients with normal cytogenetic status and its significant correlation with NF-κB expression provides a potential clue of the evasion of immune surveillance in cytogenetically normal MALT lymphomas.

PP-62

Use of Latest Genomic Techniques for Efficient Animal Production in Pakistan

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Large and small ruminants are major contributor of agriculture GDP in Pakistan providing milk, meat, skins, hides, bio fuel, and work force to millions of people in the country. There is dire need to use the attest molecular biology and biotechnological approaches to explore the hidden potential of our indigenous animals to get maximum benefit of their superior and unique genetic makeup. In most of the developing countries including Pakistan where the accurate animal production records are lacking the latest biotechnological tools like whole genome sequencing using high throughput technologies, genome wide association studies, genotyping by sequencing, epigenetic studies, microRNAs expression profiling, microarray technology, nutrigenetics and nutrigenomics and assisted reproductive technologies along with relevant bioinformatics tools can be used to improve the production and health of indigenous animals with comparatively high certainty in comparatively shorter period of time. In Pakistan we started applying such techniques on different livestock species and have done some work that resulted in useful and unique information that can be used efficiently to enhance the animal productivity in future.

Antiviral Activity against New Castle Disease Virus, Infectious Bronchitis Virus and Infectious Bursal Disease Virus from Ten Selected Cholistani Plants

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In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. An upward trend has been observed in the research on herbs and herbal products. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species. Present study is based on evaluation of antiviral potential of methanolic extracts of *Suaeda fruticosa*, *Solanum surattense*, *Oxystelma esculentum*, *Achyranthes aspera, Panicum antidotale, Sporobolos ioclados, Ochthochloa compress, Haloxylon recurvum, H. salicorn, Neurada procumbens.* These plants are well reported for their antibacterial, antifungal, anticancerous, antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepatoprotective, anti-allergic and various other important medicinal properties. The whole plant is used for methanolic extract. According to literature these plant are rich source of phytochemical and pharmacological compounds. But their antiviral activity especially against poultry pathogens like New Castle Disease Virus (NDV)-Lasoota strain, Infectious Bursal Disease Virus (IBD)-vaccinal strain and Infectious Bronchitis Virus (IBV)-vaccinal strain is need to be checked and not well reported before. In this study antiviral compounds from dried plants are extracted in methanol and later concentrate the extract by using rotary evaporator. The drugs (methanolic extracts) are dissolved in distilled water and filtered through 022 syringe filters. Viruses are propagated into 9-11 embryonated chick eggs and their ELD_{50} is calculated. To check antiviral activity the drug is mixed with live virus in varying concentrations and propagated into 9-11 days embryonated eggs. Eggs are opened after 48 hours in sterile conditions and allantoic fluid is collected. The decrease in viral load in comparison to negative control provides the expected results. According to most recent results, no. of plants are effective in controlling New Castle Disease- commonly know as Rani Kheet, on HA scale. IHA method is standardized to quantify IBD virus and screening against this virus is currently in process.

PP-64

Reverse Transcriptase Polymerase Chain Reaction: An Effective Tool for Detection and Typing of Foot-and-Mouth Disease Virus

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Foot-and-mouth disease is economically important viral disease of livestock. Rapid laboratory diagnosis and epidemiological investigation are two basic requirements for the control of this disease. The use of the polymerase chain reaction method (PCR) to amplify specific nucleic acid regions offers possibility of virus detection and typing. Primers designed from 5' UTR, 1D and 2 B regions of foot-and-mouth disease viral genome that had been characterized previously elsewhere, were evaluated for the detection of virus serotypes circulating in and around Faisalabad by reverse transcriptase polymerase chain reaction. This study evaluated these primers on clinical samples of various types including blood, saliva, tongue epithelium, and hoof tissue. Consensus primers were good at detection and serotype specific primers demonstrated appropriate specificity. The results show that the primers can be used for the diagnosis and serotyping of FMD viruses from the clinical samples collected during febrile phase of infection.

PP-65

Pathogenecity and Biochemical Characterization of Xanthomonas *axonopodis* pv. *citri* Isolated from Low Seeded Kinnow

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Invasion of citrus canker, incited by *Xanthomonas axonopodis* pv. *citri* (Xac), in the citrus orchards is the major limitation and hindrance factor for citrus industry. Despite intensive studies on epidemiology and management of this pathogen, there is little known about the biochemical characteristics of local isolates of *Xac* which may prove helpful in pathogen as well as strains and races identification. For this purpose, *Xac* local isolates were characterized through pathogenecity and biochemical assays. Diseased samples of Low Seeded Kinnow infected by *Xac* were collected from NIAB orchards. The isolates were subjected to pathogenecity test on Low Seeded Kinnow (*Citrus reticulate*), orange (*Citrus sinensis*) and lime (*Citrus limon*) by detached leaf assay and found to be pathogenic but varied in terms of disease severity. The isolates were also subjected to different biochemical tests for biochemical characterization and found to be negative for Gram's

Staining, Kovac's oxidase, Arginine dihydrolase and Fluorescent pigment test. Starch Hydrolysis, Tween 80 Hydrolysis, KOH, Catalse and Gelatin Liquefaction tests were found to be positive for bacteria. The above mention test confirms the presence of *Xac* in Low Seeded Kinnow orchards of NIAB. There is still need of DNA fingerprinting to determine the races and strains of *Xac* invading the recently developed low seeded cultivar of Kinnow. This study is helpful for biochemical and pathological identification of *Xac* isolates.

PP-66

Expression Analysis of Cellular Genes *HMGR and FAS* Involved in Steatosis in HCV Patients of Genotype 1a and 3a

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Hepatitis C virus (HCV) has become a major threat for global health. HCV is genetically highly variable and exist as different genotypes and subtypes the severity of effect within subtypes of a major genotype was observed different. So it is necessary to find the role of HCV genes of different genotypes in HCV induced pathogenesis. HCV induced Steatosis, triglyceride accumulation in hepatocytes, is the most frequent cause of abnormal liver function. Cellular genes FAS and HMGR which are involved in the Fatty acids synthesis have been reported to be activated in HCV induced steatosis. To unfold the effect of HCV of different genotypes, we studied the expression level of cellular genes, mRNA expression of genes *FAS* and *HMGR* was increased in HCV 3a patients as compared to HCV 1a patients. RNA was extracted from the whole blood. cDNA was formed from the RNA by RT-PCR. Cellular gene expression analysis was done by using specific-primers of cellular genes on BIO-RAD iQ™5 Multicolor Real Time PCR Detection System. There is an enhanced expression of steatosis inducing cellular *FAS* and *HMGR* genes in HCV patients of genotype 1a and 3a. And these genes are expressing more in patients of HCV genotype 3a then the patients of HCV genotype 1a.

PP-67

Overexpression of WRKY Transcription Factors Leads to Enhanced Resistance against Sugar Beet Cyst Nematode Heterodera Schachtii in Arabidopsis

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Cyst nematodes induce feeding sites called syncytia in the roots of their host plants which are their source of nutrients throughout their life. A transcriptome analysis of syncytia

induced by the beet cyst nematode Heterodera schachtii in Arabidopsis roots has shown thousands of genes upregulated or downregulated. Among the downregulated genes are many which code for defense-related proteins and transcription factors. One gene family which is strongly downregulated codes for the WRKY transcription factors. The Arabidopsis genome includes around 60 genes annotated as WRKY transcription factors which are involved in a variety of biotic and abiotic stress responses. So, it was hypothesized that nematodes have suppressed the defense mechanism of the plant. Expression of four WRKYs, WRKY6, WRKY11, WRKY17 and WRKY33, was studied with RT-PCR and a promoter::GUS line. The WRKY33 and WRKY6 were overexpressed constitutively using CAMV 35S promoter and WRKY33 was also overexpressed in syncytia by syncytia specific promoters Pdf2.1 and Miox5. Overexpression lines from WRKY6 and WRKY33 showed enhanced the resistance against H. schachtii which was seen by a lower number of nematodes developing on these plants as well as smaller syncytia and smaller female nematodes. However, T-DNA knock-out mutants of all these WRKYs resulted in increased susceptibility against H. schachtii. Additionally, camalexin pathway mutant pad3-1 was also challenged with nematodes which also showed susceptibility. Moreover, Mitogen-Activated Protein Kinase Kinase 4 (MKK4) which is involved in WRKY33 induced camalexin production was also overepressed and its overexpression showed increased resistance against H. schachtii. Overexpression of WRKY33 also resulted in more transcript level of camalexin gene PAD3 in syncytia as well. Our results showed that H. schachtii infection is accompanied by a downregulation of most of the members of WRKY gene family. It seems likely that the nematodes use effectors to actively downregulate the expression of these and other defense-related genes to avoid resistance responses of the host plant. Enhanced resistance of WRKY33 and MKK4 overexpression lines seemed to be due to enhanced camalexin production in syncytia which is very important defense mechanism of the plants.

PP-68

Indigenous Plants – Most Neglected Asset of Pakistan

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Treatment with plants is as old as the history of mankind. Pakistan is enriched with variety of medicinal plants. This mode of treatment is cheapest, culturally friendly and well adopted in Pakistan. Unfortunately, this empirical knowledge has been neglected and it needs to be testified on modern scientific ground for validation. The objective of the current study was to evaluate the anti-tick, anthelmintic and anticoccidial activities of a herbal formulation based on leaves of *Azadirachtaindica* and*Nicotianatabacum,* flowers of

Calotropisprocera and seeds of *Trachyspermumammi*. The herbal formulation demonstrated anti-tick activity by inhibiting the egg laying, larval mortality and reduced tick intensity/infestation on animals. Anthelmintic activity of herbal formulation was evident from the in vitro mortality of Haemonchuscontortus, ovicidal effects in egg hatch test and fecal egg count reduction in sheep naturally parasitized with gastrointestinal nematodes. Anticoccidial effects of herbal formulation were confirmed by reduction in the oocyst counts in feces, oocyst scores, bloody diarrhea and FCR in chicks treated with herbal extracts compared with infected unmedicated chicks. The survival rate and weight gain was higher in chicks treated with herbal extract compared with infected unmedicated chicks. Interestingly, values of some parameters were either comparable or even better than those of amprolium treated and/or uninfected unmedicated chicks pointing to some growth promoting factors in the herbal extract. The herbal formulation seems promising as a broad spectrum antiparasitic. Large scale controlled studies are, however, recommended for standardization of the doses and applications of the product. Studies on fraction based activity of formulation will be useful in identifying the active principles leading to development of a refined product with better antiparasitic efficacy.

PP-69

Transcriptional Regulation of PIN Mediates Root Gravitropism in *Arabidopsis thaliana*

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Auxin has been established as a key player in the regulation of plant growth and development and in responses to environmental changes. In recent years, it became evident that post-transcriptional regulation of PIN auxin efflux carrier proteins is crucial for a wide range of growth processes, specifically via determination of protein localization and steady-state levels. Substantially less is known however, about molecular determinants that control the transcription of PIN genes. This study aimed at the characterization of cis- and trans-acting determinants that control auxin responsiveness of PIN transcription. Auxin Response Factor (ARF) genes were identified as a gene family that controls expression of auxin-inducible gene via binding to canonical AuxRE elements found in a range of promoters including those of PINs. Genetic analysis of higher order mutant combinations suggested epistatic interaction between activator ARFs and repressor ARFs. Phenotypic analysis of selected arf and pin mutant combination and qPCR further highlighted potential cross talk between ARF and PIN genes. Evidence for a direct interaction between PIN promoters and ARFs, is provided by EMSA demonstrating specific binding of GST-ARF fusion proteins to AuxREs in the PIN2 promoter. Finally, site directed

mutagenesis revealed the biological relevance of such interaction by highlighting the role of redundancy and assortment of these AuxREs at a certain promoter. Overall these findings, for the first time, demonstrate a critical role for transcriptional regulation of PIN genes, in modulating adaptive growth responses in higher plants

PP-70

Exploring The Diverse Rice (*Oryza sativa L*) Germplasm on Various Genotypic and Phenotypic Traits under Stress Condition

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The study aims that exploring the germplasm potential on the basis of various Genotypic and Phenotypic traits in rice. For this purpose 30 diverse germplsm lines were collected from RRI (Rice Research Institute) Kala Shah Kaku, Lahore and USDA (United States Department of Agriculture. Various seed morphological traits were studies i.e. seed length, seed width, seed thickness, length width ratio, thousand seed weight, germination percentage, plumule length, radical length, fresh and dry weight of seedling. On the basis of these morphological traits potential of different rice lines were compared and evaluated for further screening. Twenty different SSR markers were used to evaluate the natural potential of germplasm in relation to their genetic divergence. The overall objective of the study was to screen out the diverse germplasm on basis of desirable genotypic and phenotypic traits under stress condition for the development of new rice varieties to strengthen the economy of country. The study increases the value of some germplasm by showing their good results in physical and molecular analysis. So there is high need to introduce these lines for commercial cultivation and apply it in the field to get maximum benefits with minimum loss and inputs.

PP-71

Cloning, Expression, Purification and Structural Studies on PTS Transporter II-B Protein of Methicillin-Resistant *Staphylococcus aureus* MRSA252

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Methicillin resistant *Staphylococcus aureus* (MRSA) causes serious and life threatening infections in humans. It is responsible for over 60% of *Staphylococcal* infections reported in Pakistan. Resistance against β-lactam antibiotics is increasing rapidly due to wide spread use of board spectrum antimicrobial agents. As a result, protein targeting based approach is being used as an alternative to antimicrobials. Transport proteins play an important role

in survival of bacteria. Identification of such proteins as drug targets open a new dimension towards molecular based drug design. The phosphotransferase system (PTS) is a unique system used by bacteria for sugar transport. PTS Transporter IIB is a membrane bound protein which catalyzes the phosphorylation of glucose into glucose-6-phosphate, thus preventing intracellular loss of glucose and generating a concentration gradient inside the cell. Inactivation or suppression of the PTS components can cause marked retardation of sugar uptake, its intracellular gradient maintenance and metabolism thus causing cellular growth retardation. Therefore, purification and characterization of this protein is strategically important for the treatment approaches. During current study, gene sequence of PTS transporter II-B protein of MRSA252 was cloned in expression vector pET25b and transformed in *E. coli* DH5α cells. After screening for positive clone *via* colony PCR and confirmation of insert integrity by DNA sequencing, plasmids from positive clones were transformed in *E. coli* BL21 (DE-3) cells for expression by induction through IPTG. The expressed protein was purified by ion-exchange and size exclusion chromatography by using AKTA Purifier FPLC system. CD-spectroscopy was performed which showed secondary structural elements, whereas ^{1}H-NMR, ^{1}H,^{15}N-HSQC and triple resonance NMR experiments were performed on Bruker 600MHz NMR spectrometer, coupled with cryogenic probe.

PP-72

Genetic Predesposition of Different Types of Cancers in Pakistani Population

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Dysregulation of apoptosis plays a crucial role in carcinogenesis. Cancer is a life threatening complicated disease that arises because of wide-ranging environmental and cellular factors. These external and internal stresses disrupt the spatio-temporally controlled mechanisms of the cellular signaling. Accumulating evidence suggests that signal transductions are misrepresented in carcinogenesis is reported to be involved in causing different cancers. Although there is considerable evidence emphasizing the relationship between FGFR4 (G388R), TRAIL mutation and carcinogenesis however rapidly accumulating data cannot be extrapolated to Pakistani population due to intra- and inter-ethnic variability. Prostate adenocarcinoma (PCa) is one of the leading causes of cancer related mortality in men. Genetic alterations associated with PCa are incompletely characterized and thus only limited knowledge is available about the associated functional SNPs. Pro72Arg (CGC to CCC in exon 4), rs1042522 is a common variant (CGC to CCC in exon 4) of p53 and is differentially distributed among different world populations. Tumor necrosis factor-related apoptosis inducing ligand stimulates the extrinsic apoptotic pathway by binding to death receptor 4 (DR4). Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF cytokine family, which mediates

programmed cell death (apoptosis) selectively in tumor cells. Thus, genetic alterations within the candidate tumor suppressor gene DR4, Pro72Arg (CGC to CCC in exon 4), and mutation G388R of FGFR4 genes would be expected to provoke a deficient apoptotic signaling thereby facilitating the development of cervical cancer, Prostate adenocarcinoma (PCa) and lung cancer respectively. The aim of this study is to perceive the association of Thr209Arg (C626G) polymorphism in the extracellular domain of TRAIL receptor DR4 in cervical cancer patients, G388R mutation in FGFR4 gene in Lung Cancer, and Pro72Arg mutation of p53 in prostate cancer diagnosed in local population in Pakistan. The variants were genotyped in series of cancer cases and control subjects from Pakistan, determining their impact on cervical, Prostate and Lung cancer risks. DNA was extracted using standard organic methods. PCR-RFLP analysis was done for polymorphism gene using site specific primers and restriction enzyme. The results were statistically evaluated in SPSS14. On the basis of this systematic study it is hoped that differences in allelic frequencies in different population associated with a large number of health problems can provide a valuable foundation for unhiding the mechanism of complex diseases like cancers.

PP-73

Glycine-Betaine Association with SSR Markers for Drought Tolerance in Hexaploid Wheat (*Triticum aestivum L*) Germplasm

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Plant osmotic stress responses are associated with changes in gene expression due to environmental stresses like drought, salinity and low temperature. Present study focused the DNA fingerprinting of wheat germplasm of different genotypes viz Local Genotype, Mapping Population, Synthetic and NIBGE on the basis of polymorphism. Biochemical estimation of Glycine-betaine was done by HPLC method. Root/Shoot ratio was enhanced in the drought affected genotypes. GB promoted a positive effect in wheat germplasm under drought stress. For DNA fingerprinting studies, 45 SSR primer pairs of Ksum series were tested for polymorphisam among different genotypes. The dendrogram results have shown the genotype association with the levels of GB during induced drought stress. The relationship between pattern of drought responsive biochemical attributes and DNA markers in the selected wheat genotypes was established with recommendation to select drought tolerant genotypes for sowing in drought affected areas.

PP-74

Cloning and Characterization of NHX1 Gene from Halophyte Grass Leptochloa fusca for Drought and Salt Tolerance

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Abiotic stresses such as salinity and drought have adverse effects on plants. In the present study, a novel Na+ /H+ antiporter gene homologue (LfNHX1) has been cloned from a local halophyte grass (Leptochloa fusca). The LfNHX1 cDNA contains an open reading frame (ORF) of 1623 bp that encodes a polypeptide chain of 540 amino acid residues. The LfNHX1 protein sequence showed high similarity with NHX1 homologs reported from other halophyte plants. The overexpression of LfNHX1 gene under CaMV35S promoter conferred salt and drought tolerance in tobacco plants. Under drought stress, transgenic plants showed higher relative water contents (RWC), photosynthetic rate, stomatal conductance and membrane stability index (MSI) as compared to wild type plants. More negative value of leaf osmotic potential was also observed in transgenic plants when compared with wild type control plants. Transgenic plants showed better germination and root growth at 2 mg/L Basta and 200, 250 mM of Sodium Chloride.

PP-75

Characterization of *Leptochloa fusca* H+-Pyrophosphatase Gene by Structure Prediction and Homology Modeling

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H+-pyrophosphatase is transmembrane protein involved in establishing proton motive force for active transport of Na+ across membrane by Na+/H+ antiporters. A full length Leptochloa fusca H+-pyrophosphatase was isolated and sequence was used for characterization using bioinformatics tools. Various important potential sites were predicted by PROSITE webserver. Primary structural analysis showed LfVP1 as stable protein and Grand average hydropathy (GRAVY) indicated that LfVP1 protein has good hydrosolubility. Secondary structure analysis showed that LfVP1 protein sequence contains significant proportion of alpha helix and random coil. Protein membrane topology suggested the presence of 14 transmembrane domains and presence of catalytic domain in TM3. Three dimensional structure from LfVP1 protein sequence also indicated the presence of 14 transmembrane domains and Hydrophobicity surface model showed amino

acid hydrophobicity. Ramachandran plot showed that 98 % amino acid residues were predicted in the favored region.

PP-76

Molecular Characterization of Local isolates of Staphylococcus aureus on the Basis of 16S rRNA from Poultry and their Transmission to Humans

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Staphylococcus aureus is a widely distributed throughout the world and makes up the normal bacterial flora of skin and mucous membranes of man and animals. It is involved in suppurative wound infections in man and animals. Poultry industry has also been affected by S.aureus and causing great economic and health problems. The focus of the microbiology is to correctly identify S.aureus for the treatment of the animals. Molecular biology and biotechnology is proving a helping hand in the accurate identification of microorganisms through sequence analysis of 16S rRNA gene. The aim of this study is the molecular characterization of S. aureus from poultry and poultry farm workers through 16S rRNA analysis. Bacteria were collected from poultry and poultry farm human workers. All the samples were cultured and tested biochemically. In addition, PCR amplification of 16S rRNA was performed in order to sequence the gene and further analyses through bioinformatics tools were performed. Aims and Objectives: 1. Molecular characterization of S.aureus in poultry and humans through 16S rRNA sequencing. 2. Finding the phylogenetic relationships among S.aureus isolates. 3. Detection of zoonoses between poultry and human. Results and Conclusion: We amplified 16S rRNA gene with PCR primers and the sequence was compared with NCBI database reported S. aureus sequences. Resemblance was found between human and chicken isolates. Phylogenetic analyses were performed by using MEGA 5.1 software that also showed phylogenetic relationship among them.

PP-77

Development of Subunit Vaccine against Hydropericardium Syndrome using Adenoviral Recombinant Proteins

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Hydropericardium syndrome (HPS) is a disease of poultry that is caused by fowl adenovirus-4. Inactivated liver homogenate from diseased birds is still the choice of vaccine in some countries which disseminates numerous pathogens along with inactivated virus. Moreover incomplete attenuation or inactivation, reversion to virulence and the oncogenic potential/ genetic instability of the adenoviruses have prevented their use in routine vaccines. To address this issue an effort is made to develop a subunit vaccine. For

this purpose 100K, penton base and short fiber proteins of HPS virus was expressed in *Escherichia coli* and used as subunit vaccine in broilers. Immunogenecity of the recombinant proteins and challenge protection test against pathogenic virus demonstrated the ability of recombinant penton base protein to confer 90% protection. Results suggest that the recombinant penton base protein is a candidate for subunit vaccine against HPS.

PP-78

An Efficient Protocol for Rapid In-vitro Multiplication of Carnation *(Dianthus caryophyllus)*

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Among the ornamental cut flower, Carnation has its own aesthetic value. The study is conducted to generate efficient invitro protocol through micropropagation. Seeds were purchased from commercial grower. Seed were sterilized in different concentration of Clorox solution (2%, 5% 10% and 15%). Best results of sterilization were obtained at 10% Clorox solution. For culture initiation, sterilized seeds were cultured on Murashiage and Skoog basal medium without any growth hormone. The cultures were maintained at 25+2°C and maintained at light intensity of 2500-3000 lux with photoperiod of 16 hours per day. After four weeks invitro nursery was raised. For invitro multiplication shoot tips (meristem, nodes and internodes) were cultured on MS basal medium supplemented by BAP (at 0.2, 0.5, 1.0 and 1.5mg/l) and IAA (0.2, 0.5 and 1.0 mg/l). Best results were obtained on MS supplemented by 0.5 mg/l and 0.1mg/l IAA. Apical meristem showed pronounced effect for shoot multiplication than nodal explants. Multiple shoots were obtained from apical shoot tip after four weeks of sub culturing.

PP-79

The Evaluation of Medicinal Potential of Different Extracts of *Justicia californica*

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Justicia californica, commonly known as humming bird bush, is a species of flowering shrub native to the deserts of southern California, Arizona, and northern Mexico. Other member species of the genus Justicia have been reported as antibacterial, ant diabetic, antifungal, anti-oxidant and other medicinal activities. Justicia californica was selected for preliminary screening for its medicinal potential. It was found that the plant is rich in phytochemicals and exhibited anti-bacterial activity against gram positive (S. aureus) as well as gram negative bacteria (P. aeruginosa and E. coli). In addition it is effective anti-fungal against A. niger¸ F. fumigatus and F. solani. Its free radical scavenging ability is also elucidated by

DPPH and reducing power assays. Its dilutions 100 ppm and 10 ppm exhibited DNA protection ability against free radicals. Furthermore the cytotoxicity effects are also observed by using brine shrimp assay. This initial study on plant has proved that like other sister species plant has potential medicinal and pharmaceutical importance.

PP-80

In vitro Regeneration of Groundnut (*Arachis hypogaea L*) under Various Hormonal Regimes

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Present research was focused to use tissue culture techniques for the optimization of groundnut regeneration system with the need to strengthen the awareness of what advantages biotechnology can offer to the environment and food security. An efficient and reproducible tissue culture system was established for two groundnut varieties; Golden and BARI 2001 to achieve best regenerable callus induction, multiple shoots induction and efficient root formation, using embryo slices as explants. Explants were aseptically grown on Murashige and Skoog (MS) medium supplemented with various applications of BAP individually or in combination with NAA and IAA to induce callus culture under suitable condition. The highest callus induction frequency i.e. 86% (Golden) & 78% (BARI 2001) was achieved on MS media fortified with 5.5 mgL^{-1} BAP and 1.5 mgL-1 NAA. The highest multiple shoot induction frequency (86% & 78%) was obtained on MS media with the application of BAP (4 mgL-1) along with NAA (1 mgL-1) and TDZ (1.1 mgL-1), with highest multiple shoot/ plant (9 & 6) and maximum shoot length (4.8 & 4.1 cm) in Golden and BARI 2001. Multiple shoots were separated aseptically from the bunch and placed on MS media, fortified with 1 mgL-1 filter-sterilized IBA to induce the roots without the application of any PGRs. The highest percentage of rooting (90% & 76%) with maximum roots/shoots (8.3 & 6.2) and significant root length (8.1 & 7.0 cm) were achieved in Golden and BARI 2001, respectively. After roots formation the plants were transferred to hydroponics system, where significant root elongation occurred and plants were successfully acclimatized in plastic bags and shifted to pots in green house with 85% survival rate. Present research is a base for genetic transformation of any desirable gene in elite groundnut varieties in the future.

PP-81

Metabolic Analysis in Transgenic Halotolerant Tomato Model Systems

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Changes in metabolism occurring in homozygous lines of transgenic tomatoes with the highest expression level of *HAL I* and *HAL II* compared to control plants were studied with Electrospray Ionization (ESI) and Matrix-Assisted Laser Desorption Ionization (MALDI) mass spectrometry techniques. Principal component analysis (PCA) of the metabolic profiles obtained with ESI-MS successfully distinguished the wild type tomato cv Rio Grande seeds from the transgenic *HAL I* and *HAL II* population. Increased biomass was observed for the transgenic halotolerant tomato lines in comparison with control when germinated under 0, 50 and 100 mM salt stress conditions for a period of seven days. Multivariate Partial least square - Discriminant analysis (PLS-DA) revealed metabolic phenotypes and differences in seedlings before and after salt stress for each plant population. Based on mass, sugars (Sucrose, glucose), organic acids (cinnamic acid, malonic acid, chorismic acid) and fatty acids (Linolenic acid and palmitic acid) were putatively identified as the metabolites responsible for discriminating the transgenic from the wild type populations with or without salt stress. Tandem mass spectrometry confirmed the identity of the metabolites varying in amount between samples. MALDI-mass spectrometry was used to analyse the distribution of metabolites direct within the plant tissue of transgenic and wild type tomato seeds. The MALDI-MS procedure was optimized for matrix application, laser energy and frequency and machine quadrapole settings. Only a few masses with insufficient counts to map as images across tomato seed sections were obtained. Metabolomic studies revealed distinct metabolic phenotypes of halotolerant transgenic tomato lines and provided biochemical indicators possibly involved in protecting the transgenic salt tolerant lines from salt damaging effects.

PP-82

Hepatitis C Virus Genotypes in Twin Cities (Rawalpindi/Islamabad) of Pakistan

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Hepatitis C is one of the major infectious diseases and about 10 million people are infected with Hepatitis C virus (HCV) in Pakistan. This study was conducted to determine the HCV genotypes in the patients of hepatitis C in Rawalpindi and Islamabad region. A total of 295 individuals with age range 17-75 were registered for HCV genotyping at Islamabad Diagnostic Centre, Islamabad during the period of 2008-2011. All samples were tested with real time PCR using Qiagen assay (USA) to determine HCV viral load. In HCV positive samples, PCR amplified products were used for genotyping using Invader HCV genotyping assay (Third wave technology, USA). Out of 295 subjects, 227 (77%) were females while 68 (23%) were males. Among 295 cases, HCV was detected in 244 (83%) individuals. All HCV positive samples were tested for genotyping, of which type 3 was 91%, type 1 was 6%, type 4 in 2% and type 2 was identified in 1% of cases. In females, HCV type 3 was 89%, type 4 was 1%, type 1 in 9% and type 2 was identified in 01 patient only. On the other hand, in male subjects, 98% had type 3 while genotype 2 was detected in 1 patient. HCV genotype 4 was also found in 1 patient while genotype 1 was not detected in male subjects. Current study supports previous data that type 3 is the most common HCV genotype in Pakistan. These findings also highlight the importance of genotyping before starting the interferon therapy as other types also exist.

PP-83

Comparative Analysis of Bioactivities of Peel Extracts of Five Citrus Species of Pakistan

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Pakistan is among the top ranked producer of some citrus species. The five important species of the genus cultivated in Pakistan are: Citrus reticulate (Kinow), Citrus sinensis (Sweet orange), Citrus limon (Lemon), Citrus limetta (Sweet lemon) and Citrus aurantium (Bitter orange). These fruits are used in different food processing industries and these industries are producing bulk waste in form of peels of citrus fruits. Keeping in mind the idea of biological waste utilization, citrus peels are used in present study to identify their biological activities. Qualitative as well as quantitative phytochemical tests of methanolic extract (ME) of peels of citrus species revealed the presence of active compounds in the samples. Then ME were tested for different bioactivities viz antibacterial, antifungal, anti-oxidant, DNA protection and cytotoxicity. The methanolic extract of all peels have shown significant antimicrobial activity against bacterial and fungal strains used in this study but Citrus limetta proved to be most effective among all tested species with MIC of 10 µg/mL and MBC of 50 µg/mL against E. coli. For antioxidant C. reticulata and C. aurantium have IC50 of 4.8 µg/µL and 4.4 µg/µL respectively proving these to be rich in antioxidants. Based on this preliminary data it is concluded that peels of these citrus fruits have pharmaceutical

potential which needs to be exploited in future studies to obtain plant derived natural drugs.

PP-84

Identification of Therapeutic Drug Targets for *Streptococcus sanguinis* SK36

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The main focus of this study is to employ 'Subtractive Genomics Approach' on strain specific available genome or proteome for the identification leading to characterization of strain specific drug targets. Screening of specific drug target has paved a way to explore the potential drug targets and opened up a new horizon of computational modeling. This approach is carried out with comparative analysis of *Streptococcus sanguinis SK36*. The protocol followed for the purpose comprise of various bioinformatics' tools and databases. The protocol started with similarity search between host and pathogen proteome leading to study of non-homologous essential proteins using database of essential genes. The essential non-homologous genes were studied for their metabolic function and their association study using Kyoto Encyclopedia of Genes and Genomes Database (KEGG), the sub-cellular localization analysis of all essential genes was done using Psrotv, the drug targets were identified using drug bank database. Additionally, SVMProt server was utilized to characterize the identified non homologous hypothetical essential proteins on the basis of family characterization. The potential for each of the identified drug targets were also evaluated using Drug Bank database. The followed protocol leads us to the identification and characterization of non-homologous essential proteins which were non-homologous to the host genome. These screened out non-homologous essential drug targets which were four in number ensure that survival of pathogen is dependent on them and hence they can be targeted for drug discovery/ investigated as therapeutic drug targets.

PP-85

Determination of Anti-HEV Seroprevalence and Proogesterone Levels in Pregnant Women Population of Low Socio-Economic Status

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Hepatitis E virus (HEV) is a major public health problem in the developing countries including Pakistan. HEV infection in pregnant women is more common and fatal in the later stages of pregnancy. The mortality rate due to HEV-induced hepatitis is as high as 15-20%.

The present study was designed to determine the sero-prevalence of subclinical HEV infection and progesterone levels in pregnant women of low socio-economic status. A total of 90 pregnant asymptomatic healthy females were included in the study. Prevalence of anti-HEV antibodies and progesterone levels were determined by enzyme linked immunosorbent assay (ELISA) kits. The overall prevalence of sero-positive HEV-IgG was 60% and IgM was 13.3% among these randomly selected pregnant women. The antibodies were widely distributed among all age groups and trimesters. A higher trend in sero-positivity for IgG and IgM was observed with the increase in trimester and age. Alteration of levels of progesterone from normal level was observed in all the trimesters with high levels observed during the first trimester and extreme low levels during the 2nd and 3rd trimester. Levels of progesterone were found to be higher ($P < 0.001$) in HEV IgM positive pregnant patients when compared to HEV IgG positive patients. Poor nutritional and environmental conditions appear to be potential risk factors associated with high HEV sero-prevalence and alterations in the normal hormonal level observed in pregnancy. These alterations may serve as a reason for high mortality rate seen in HEV positive pregnant females.

PP-86

GSTM1 and GSTT1 Gene Deletions in Cancer

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Cancer incidence has been increasing at an alarming rate in last few decades. One of the main reasons of this increase has been attributed to genetic mutations. Carcinogen detoxifying genes play an important role in detoxifying the carcinogen and a mutation in these genes might contribute towards cancer initiation or progression. The current study is aimed at evaluating the role of two genes involved in phase II carcinogen detoxification: GSTM1 and GSTT1 genes. These genes have been known to show deletion polymorphisms. Normal as well as cancer individuals show these deletions. It was found that the genetic deletions of GSTM1 and GSTT1 genes have been found to be significantly associated with head and neck cancer and nonsignificant association with breast cancer. However the exact trends of these deletions in different cancer can be evaluated with a more detailed study with larger sample size.

PP-87

Null Association of FTO Gene Polymorphism with Type 2 Diabetes Mellitus in Pakistan

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Type 2 Diabetes mellitus is a serious metabolic disorder which has become a serious threat to the health and economy of the world. The uncontrolled glycemic profile of diabetes patients puts them at an increased risk of diabetes associated disorders like obesity, microvascular and macrovascular complications. Molecular research has shown involvement of crucial single nucleotide polymorphisms (SNPs) in genes which are associated with insulin imbalances. SNPs in FTO gene has been reported to be associated with the risk of diabetes mellitus and obesity. Here some SNPs in the FTO gene have been studied for association with T2DM in Pakistan. The results did not show any significant association of the SNP with T2DM. This study concludes that the SNP in the FTO gene is not associated with T2DM in Pakistan.

PP-88

Assessing Tomato Rhizospheric Bacterial Diversity (Pishin District) Pakistan

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Identification of rhizospheric soil bacteria always is a primary step in discerning quality/suppressive state of soil, in deciphering species with plant growth promoting potential and also any pathogenic bacterial factor. Tomato agriculture field located in Pishin district hold economical importance to local farmers and this field remains unexplored in terms of delineating microbial population. 16S ribosomal RNA (16S rRNA) gene sequences are most commonly used in microbiology to confidently identify bacteria. 16S rRNA gene is universal among domain Bacteria. It is highly conserved, enough to establish homology and has considerable amount of variation among different species to discriminate and identify organism. Especially from environmental samples where metabolic variation is unable to resolve solely a true picture of bacterial species residing. This study was carried out to characterize rhizospheric bacteria of Tomato agriculture field. Seven isolates were successfully isolated. Six belonged to genus *Bacillus* and one to genus *Paenibacillus* based on 16S rRNA gene sequence similarity. Among the isolates belonging to genus *Bacillus*, isolate A2 and A13 were placed close to *B. pumilus* with 95% and 99% sequence identity respectively. Isolate A3's closest phylogenetic relative was *B. subtilis* with 99% sequence identity. Isolate A4 with 99% sequence identity was placed closest to *B. anthracis*. Isolate A27 was placed close to *B. sonorensis* with 99% sequence

identity. Isolate A35 with 95% sequence identity was placed closest to *B. atropheus*. One isolate A18 having 95% sequence identity was placed close to *P. polymyxa*.

PP-89

Prolactin Gene Polymorphism in Nili-Ravi Buffaloes in relation to Sahiwal and Achai Cattle

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In this study, prolactin gene polymorphism was investigated in Nili- Ravi buffaloes, Sahiwal and Achai cattle breeds, 100 per group, using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique. Only genotype GG was observed in the case of Nili-Ravi buffaloes. In Sahiwal and Achai cattle, three genotypes were found, AA, AG and GG: the frequencies of these genotypes were 72%, 18% and 10% in Sahiwal cattle and 44%, 34% and 22% in Achai cattle, respectively. The frequency of genotype AA was found to be higher in both cattle breeds. Results of chi-square test at $P < 0.05$ revealed that animals of Achai cattle were in Hardy–Weinberg equilibrium, whereas Sahiwal cattle were found to be deviating.

PP-90

Alteration in Serum Lipid Profile Patterns in Prostate cancer – Quest for an Optimal Biomarker

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The biomarkers of Prostate cancer, currently used, are sub-optimal. For example, Prostate specific antigen (PSA) is most widely used but it is controversial because of its specificity and sensitivity. A role of high levels of serum cholesterol and some specific fatty acids in prostate cancer incidence and progression has been suggested by a number of epidemiological and preclinical studies. High fat/cholesterol diets have also been linked to PCa incident and progression in some reports. The focus of the present study is to determine the serum lipid species associated with the risk of prostate cancer and their correlation with each other among Asian men. In this hospital based study, fasting venous blood was collected from patients with prostate cancer and analyzed for triglycerides, total cholesterol, high density lipoproteins (HDL), glucose and LDH using standard kit methods while low density lipoprotein (LDL) and very low density lipoprotein (VLDL) was calculated using Fried Wald's formula. Consumption of high dose of animal fats (cheese,

yogurt and red meat), alcohol, cigarettes and tomato based products was also taken into consideration in order to determine the relationship between dietary habits and incidence of prostate cancer. This information could be important in understanding the possible application of serum lipid profiles in cancer diagnostics.

PP-91

Detection of Rieske [Fe_2-S_2] Center of Hydrocarbons Degrading Bacteria from Oil Contaminated Soil: A PCR Based Approach

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In the existing world of immense commercialization, contamination of soil with crude oil or its components is one of the types of soil pollution ultimately affecting agriculture of the country. Fertility of the soil gets affected by hydrocarbons contamination and the vegetation of the polluted site devastates. Hydrocarbons are a large group of organic compounds which have become the common threat to our environment as the grievous pollutants. Bioremediation of the contaminated soil via microbes is cost effective and eco-friendly way to remove these pollutants from soil and rehabilitate the agricultural land. These microorganisms could be bacteria, yeast, fungi or algae, bacteria being the ubiquitous in the environment. Bacteria possess different enzymes to degrade hydrocarbons and convert those chemical compounds into energy, cell mass and biological waste products. In general diversified hydrocarbons degrading bacterial strains with the ability to degrade multiple hydrocarbons are detected and confirmed by combining the use of selective enrichment in minimal media supplemented with hydrocarbons and molecular approaches using primers or probes to measure hydrocarbons degradation potential. The present study explored the hydrocarbons degrading bacterial community of lightly and heavily crude oil polluted soil, having low vegetation and no vegetation, respectively by detecting the presence of Aromatic ring dioxygenase expressing bacteria (ARDB) and Rieske [Fe_2-S_2] center in polymerase chain reaction.

PP-92

Response of Canola (Brassica napus L) to Externally Supplemented Calcium under Irrigated and Drought Stress Conditions

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Low availability of soil moisture is one of the most important limiting factors affecting plants growth, metabolism, yield and productivity in arid and semi-arid regions of the world. Due to the growing population of human beings, irregular and uncertain rainfall and

extensively developing industrialization, the available water resources are rapidly depleted around the world which poses greater threat to the future demand of world food. To cope with the aridity of the soil, strategies must be adopted to increase the drought resistance ability of plants and crops and to ensure better food yield under the situations of aridity and low water availability. In this experiment, canola (Brassica napus), being on of the important oilseed rapes was exposed to drought stress after treatment with externally supplied calcium in the form of calcium nitrate solutions in three alternate doses and the effects of these doses were studied on leaf relative water content, total chlorophyll content and membrane stability index as some of the common parameters used for assessment of drought stress intensity and degree. It was investigated that drought stress led to reduction in leaf relative water content, degradation in total chlorophyll content and rupture of cell membranes. Exogenously applied calcium was found to ameliorate the harmful effects of drought stress by improving the relative water content, protecting the total chlorophyll content and membrane from being destroyed by the drought stress.

PP-93

A Study on the Determination of Risk Factors Associated with Babesiosis and Prevalence of Babesia sp., by PCR Amplification, in Small Ruminants from Southern Punjab (Pakistan)

S Fatima, F Iqbal

Babesiosis is a parasitic infection due to the multiplication of tick borne parasite, Babesia sp., in erythrocytes of host, which includes a wide variety of vertebrates including small ruminants causing decreased livestock output and hence economic losses. The objective of the present study was to establish a PCR based method for the detection of Babesia sp. in small ruminant population in Southern Punjab and to determine the risk factors involve in the spread of babesiosis. A total of 107 blood samples were collected from 40 sheep and 67 goats in seven districts of Southern Punjab from randomly selected herds. Data on the characteristics of the animals and the herd were collected through questionnaires. 36 blood samples (34% of total) produced the DNA fragment specific for 18S rRNA gene of Babesia sp., by PCR amplification, of which 20 were sheep and 16 were goats. Samples from all seven district contained Babesia positive samples and prevalence varied between 18 to 68%. It was observed that male animals (P = 0.009) and young animals under one year of age (P = 0.01) were more prone to the parasite. It was observed that herds consist of more than 15 animals (P = 0.007), composed of mixed species of small ruminants (P = 0.022), associated with dogs (P = 0.003) and dogs having ticks on their bodies (P = 0.011) were among the major risk factors for the spread of babesiosis in small ruminants.

PP-94

Targeting the Threats of Multidrug Resistant Pathogens by Natural Products and Synthetic Compounds

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The emerging problem of antibiotic resistance has become a serious threat to global public health. A rapid decline in research and development of new antibiotics coincides with increasing frequency of infections which are caused by multidrug- resistant (MDR) pathogens. MDR has been becoming common in clinically important pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Bacteria displays a unique ability to adapt the changes in their environment and to develop mechanisms to protect themselves against toxic compounds The major mechanisms that cause multidrug resistance in bacteria is the active efflux of drugs, such as antibiotics from a cell. Despite efforts to limit their spread, rates of multidrug resistant bacteria continue to increase throughout the world. The present study encompasses the efforts to discover new and effective inhibitors against MDR strains of *S. aureus* and *P. aeruginosa,* that can increase the susceptibility of antibiotics. Over 1400 fully characterized natural and synthetic compounds were screened by high-throughput assay MABA. This lead to the discovery of some potent and highly active reproducible MDR inhibitors belongings from various chemical classes such as, monoterpenes, sesquiterpenes, flavonoids, quinolones, thiourea derivatives and organometallic class. Mechanistic studies on some selected compounds from synthetic and natural origin were also carried out to analyze the compound-induced effect on membrane potential, efflux pump inhibition, etc. By electron microscopic imaging, ultra structure defects in resistant cells were also evaluated. The study also includes evaluation of cell damage by superoxide, produced by selected compounds, and the reversal of multidrug resistance by using the MDR inhibitors.

PP-95

Mutational Analysis of Different forms of Leukemia found in Pakistan

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Leukemia is disease of uncontrolled proliferation of hematopoietic stem cells. These cells remain undifferentiated and have an inexhaustible potential to divide. Leukemia is of four basic types; ALL, AML, CLL, and CML. The ratio of AML is higher than all forms of leukemia, while in children ALL is most common. Most Frequent genetic aberration in AML are the mutations in flt3 gene. FLT3 ITDs are present in 20-30% of AML while FLT3 TKD mutations

are reported in 7% of patient. These mutations cause constitutive activation of FLT3 receptor. ITDs are typically present in exon 14 and 15 of this gene. In the present study occurrence of mutations in exon 14, 15and intron 14 of FLT3 was screened by PCR-SSCP and further confirmed by sequencing. Peripheral blood of 10 normal and 26 leukemia samples was collected. Out of which 9 were of ALL, 9 CML, 1APL and 5 of lymphoma patients. All these samples were subjected to DNA extraction followed by PCR and SSCP. SSCP revealed obvious differences in 4 patient samples; P7, P14, P25, and P26, which were selected for further screening by sequencing. A total of 1 normal and 4 patient samples were sequenced. It was observed that none of the patient had FLT3 ITDs but point mutations were seen in 3 of 4 patients, whose amplified exon and intron was sequenced. In 2 of patients; P25 and P26, numerous insertions, deletion and substitution mutations were observed these mutations may have an effect on progression of the disease. Their exact effect on development and prognosis of disease is yet to be determined, which can be done by expression profiling of the mutated flt3 from these patients and by increasing the magnitude of patient samples in further studies.

PP-96

Lack of Association between rs8099917 IL28B Gene Polymorphisms and Therapeutic response in Pakistani HCV Patients

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Hepatitis C virus (HCV) has infected 10% of Pakistani patients as a major cause of chronic hepatitis. Several genome based studies revealed that single nucleotide polymorphisms (SNPs) in the interleukin 28B (IL28B) region are associated with the response to current treatment among HCV-infected individuals of European, African and Asian ancestry. Different groups of researchers have reported expression of IL28 was higher in patients homozygous for the allele (rs8099917TT) as compared to other alleles which ultimately affects the overall response to therapy. However, the effect of this polymorphism has not been studied in HCV infected patients from Pakistan. A total of 148 patients with different virological response to standard therapy were included in the study. Out of total 148 patients, TT, TG and GG genotype of rs8099917 were in 79 (53.4%), 63(42.6%), 6(4%) patients, respectively. IL28B rs8099917GG was not found to be completely associated with virological response in our study population in contrast to other population based studies irrespective of the fact that only 5(15.6%) patients with GG genotype and HCV 3a genotype were non-responders out of 32 patients, while one patient with GG genotype showed an early response to therapy. Moreover, 27 (84.4%) patients out of 32 non-responders were with genotype TG/TT. In light of these observations it can be stated that IL28B rs8099917 does not represent an association with therapeutic response in our study population.

PP-97

Detection of New Emerging Subtype of Infectious Bronchitis Virus in Broiler-Breeders in Pakistan during 2011-2013

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Infectious bronchitis virus (IBV) is a major respiratory disease in chickens all over the world and can be found even in vaccinated flocks. This virus causes huge economic losses when combined with other infectious agents. The current study was designed to detect and identify the prevalent serotypes of IBV in the suspected samples. In total 120 suspected field samples from different poultry populated areas of Punjab were processed and tested for IBV usingRT-PCR. In this regard 34 samples were found positive. Moreover, subtype specific primers were designed and out of 34 IBV positive samples, 30 were typed as Massachusetts 41 (M41) serotype and 4 were typed as 4/91 subtype. This is first report regarding the circulation of IBV 4/91 subtype in poultry from Pakistan. Further molecular characterization of this virus is ongoing.

PP-98

Analysis of inhibin Alpha Gene (769 G>A) Mutation in Patients with Premature Ovarian Failure

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BACKGROUND: Premature Ovarian Failure (POF) effecting nearly 1% of the population, results due to loss of functional ovarian follicles in women less than 40 years of age. POF can manifest as absent menarche (primary amenorrhea) or premature depletion of ovarian follicles before the age of 40 years (secondary amenorrhea). The etiology of POF is heterogeneous whereas, majority of the cases are idiopathic. Known causes of permanent damage to the ovaries that could result into POF are autoimmune conditions, chemotherapy or radiotherapy, pelvic surgery, exposure to environmental toxicants and genetic causes. Biochemically, POF is characterized by low levels of gonadal hormones (Estrogens and Inhibins) and high levels of gonadotropins (LH and FSH). The glycoprotein Inhibin, functions as a negative feedback to control the FSH level which is a key determinant of follicular development. Inhibin alpha gene mutations have recently been studied in different populations with a missense mutation (769 G>A) in the Inhibin Alpha subunit gene found to be significantly associated with POF. This study was designed to

screen the INHα 769G>A mutation in Pakistani POF patients. METHODS: Blood samples were collected from the Gynecology departments of various tertiary care hospitals of Lahore. DNA extraction was done and the genotyping of INHα mutation (769 G>A) was performed by Direct DNA sequencing technique. RESULTS: Screening of the Inhibin alpha gene for 769G>A mutation in exon 2 through direct sequencing revealed a heterozygous transition in only 2% of the POF patients. CONCLUSION: Identification of inhibin gene mutation in only 2% patients that were analyzed indicates the role of this mutation in the etiology of POF is very low or it may not be associated with the disease phenotype in this group of patients.

PP-99

A Study on the Prevalence of a Tick Transmitted Pathogen, Theileria sp. in Cattle from Southern Punjab by Polymerase Chain Reaction Amplification

Sadia Shahnawaz, Maryam Ashfaq, Furhan Iqbal

Blood samples were collected from 144 large ruminants, consisting of 105 cattle and 39 bufalloes, from six districts of Southern Punjab. Data on the characteristics of animals (gender, age and absence or presence of ticks and the herd (herd size, herd composition, dogs associated with the herds and absence or presence of ticks on dogs) were collected through questionnaires. Animal gender and the presence of ticks on animals were among the major risk factors involved in the spread of theileriosis in the study area. Two different parasites detection techniques, PCR amplification and screening of Giemsa stained slides, were compared and it was found that PCR amplification is a more sensitive tool (19% parasite detection) as compared to smear scanning (3% parasite detection) for the detection of T.annulata. 28 out of 144 animals produced the 721-bp fragments specific for Theileria annulata from 5 out of 6 sampling districts. Blood and serum parameters, including glucose, hemoglobin, cholesterol, ALT, AST and LDH of calf, cattle and buffalo were measured and compared between parasite positive and negative samples. ALT, AST, cholesterol and LDH were the most significantly affected parameters in Theileria annulata positive samples, when compared to healthy ones, indicating that this blood parasite severely affects serological profile of animals and hence their output.

PP-100

Discovery of Antiglycation Agents: An Efficient Strategy for the Molecular Treatment of Diabetes

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Diabetes mellitus is a metabolic and endocrine disorder. Hyperglycemia is the hallmark of diabetes. Continuous hyperglycemic condition results in the glycation of many body proteins and finally leads to the diabetic complications. Glycation is a natural, spontaneous reaction between proteins and reducing sugars which initiates without the help of any enzyme, and results in the generation of Advanced Glycation Endproducts (AGEs). The process of glycation of proteins is not only implied to be a marker of the onset of diabetic complications, but also found to be the core reason of diabetic associated disorders. Binding of excess sugars (e.g. ribose, glucose, fructose, glyoxal and methylglyoxal i.e. reactive oxygen species) with the proteins in the living system, largely modify their structures and functions in such a manner that it damages different organs. These cellular and molecular changes can eventually become detrimental and pathogenic. Because of the fact that "glycation of proteins cannot be evaded"; therefore, there is a need for an approach to develop the compounds which have the potential to inhibit or reverse the complex reactions of protein glycation. The present study encompasses the discovery of antiglycation agents by screening over 2,000 fully characterized natural and synthetic compounds. This leads to the identification of highly active and potent inhibitors of polyphenols, cyclopeptide alkaloids, flavonoids, oxindole derivatives, benzohydrazide Schiff bases, urea derivatives and organomatallic class of compounds with no cytotoxicity. On the basis of results obtained during this study, our work represents an example of systematic and comprehensive investigation of chemical and biochemical aspects of inhibition of protein glycation. This study has identified series of potential molecules as anti-glycating agents. Further research is, however, needed in order to evaluate the therapeutic potential of compounds, identified during this study, as drugs.

PP-101

Antimicrobial Activity of *Allium sativum* under Fungal Induction

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Plants are the sleeping giants of pharmaceutical industry and provide natural source of antimicrobial drugs. Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antimicrobial agents. Different medicinal plant extracts were tested and some of them approved as new antimicrobial drugs. Allium sativum (Garlic) is of particular interest due to its therapeutic actions. Allium sativum was induced with fungus i.e. Fusarium solani (biotic stress) at seedling stage and harvested at different dpi (day post inoculation). Antimicrobial activity of different extracts (buffer, acetone, methanol) of Allium sativum was evaluated under control and biotic stress environment using the disc diffusion method against different bacterial and fungal strains. Results indicated that different extracts showed broad spectrum of activity by forming clear zones of inhibition at different dpi. The highest antimicrobial activity of buffer

extracts of Allium sativum was observed against Bacillus subtilis at dpi 5 in fungal induced extracts. Acetone and methanol extracts showed highest antimicrobial activity against A. niger in fungal induced extracts at dpi 7 and dpi 6, respectively. Minimum inhibitory concentration (MIC) of Allium sativum extracts was determined that could inhibit the growth of microbes. On the basis of these preliminary results it may be concluded that this plant is a rich source of antimicrobial compounds. In future, this study may be used for industrial scale extraction, isolation and purification of antimicrobial compounds particularly peptides/proteins which may find place in medicinal industry as constituent of antibiotics.

PP-102

Genetic Transformation of Ajuga bracteosa Wall ex Benth for the Enhancement of Secondary Metabolites

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Ajuga bracteosa Wall. ex. Benth. (Labiatae) is highly medicinal herb with enormous folk ethnobotanic usage. It possesses compounds of significant medicinal value like withanolides, ecdysteroids, irridoid glycosides etc. rol genes are potent inducers of secondary metabolism. Enhanced biosynthesis of these compounds can have a significant effect on plants' pharmacological potential. The foremost objective of the present study was to evaluate the effect of rolA and rolC genes on secondary metabolites biosynthesis. Agrobacterium tumefaciens strains LBA4404 containing either pLBR29 harboring rolA gene or pLBR31 harboring rolC genes were used for transformation. Both vectors contain NPTII as selectable marker gene. Nodal region explants were used to raise transformed plants. Molecular analysis revealed clear bands of 308, 540 and 779 bp for rolA, rolC and NPTII respectively. Transformed plants were found to have reduced leaf size, changed leaf shape and color, yellow spots on leaves, vigorous shooting and reduction in plant height. Methanolic extract of transformed plants was evaluated for TLC analysis. Six markers of highly medicinal value (Coagulansin A, withanolides F, Withacoagulin, Withanolide H, Withacoagulin E and 20-hydroxyecdysone) were detected in A. bracteosa. TLC analysis depicted that the amount of these compounds was tremendously increased by rolA and rolC genes in A. bracteosa.

PP-103

Phytochemical and Pharmacological Screening of Lespedeza bicolor Turcz (Papilionaceae)

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Anticancer activity against Human lung carcinoma (LU-1) and Human prostrate carcinoma (LnCap) along with antimicrobial and antioxidant activity on DPPH ((1,1)-diphenyl-2-picrylhydrazyl) and Hydrogen peroxide radicals scavenging activity and the contents of total phenolic and flavonoids were assessed in methanol extract of Lespedeza bicolor. The highest content of total phenolic content was detected in the arial part of Lespedeza bicolor (0.5-1.7 mg gallic acid equiv./g), while the highest content of total flavonoids was found in the aerial part of Lespedeza bicolor (0.102-0.148 mg/g D/W). Lespedeza bicolor arial parts and root extract showed IC50 value of 12.5µg/ml and 50µg/ml against human lung carcinoma (LU-1) whereas, ≤ 12.5 µg/ml and 12µg/ml were calculated against Human prostrate carcinoma (LnCap) cell line. MIC value of 20-35 µg ml−1 has been observed gainst Aspergillus fumigates, Aspergillus niger, Fusarium solani and Mucor sp in comparision with 1-2.5µg/ml of Terbinafine used as a standard fungicide. MIC value of 20 µg/ml and 35 µg ml−1 of Lespedeza bicolor arial parts and root extract against bacterial pathogen Klebsiella pneumonia and 20-50 µg ml−1 against Enterococcus has been measured. DPPH radical scavenging activity of Lespedeza bicolor with IC50 values of ≤ 50 µg/mland ≤ 200 µg ml−1 was observed whereas, hydrogen peroxide scavenging activity with IC50 values of ≤ 25 µg/ml for arial parts and ≤ 50 µg ml−1 for the root extract of Lespedeza bicolor has been shown with galllic acid (R2= 0.819) and ascorbic acid (R2= 0.728). These data suggested that the methanolic extract of Lespedeza bicolor could be potential candidates for natural antioxidants and anticancer.

PP-104

Actinomycetes Diversity of a Saline Lake and their Antimicrobial Potential against Multi Drug Resistant (MDR) Ventilator Associated Pneumonia (VAP) Causing Bacterial Pathogens

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A collection of forty actinomycetes isolated from the water and mud samples of the saline lake situated in Kalar Kahar in the salt range, province Punjab, Pakistan, was screened to investigate their potential against multi drug resistant (MDR) ventilator associated pneumonia (VAP) causing bacterial pathogens. The isolates showed strong pH tolerance and were grow in the range of pH 9-11. The taxonomic status of the selected isolates was determined by morphological, biochemical and physiological characterization as well as by 16S rRNA gene sequencing. The results revealed that majority of the isolates (90%) belong to the genus Streptomyces. The isolates exhibited noteworthy antimicrobial activity against

multi drug resistant (MDR) ventilator associated pneumonia (VAP) causing bacterial pathogens including Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli, Enterobacter and Acinetobacter spp. Additionally the isolates showed moderate cytotoxicity against larvae of the brine shrimp (Artemia salina) in a microwell cytotoxicity assay. The chemical screening of the crude extracts obtained from each of the isolate, by thin layer chromatography (TLC) using various staining reagents and by HPLC-UV, indicated an impressive diversity of the compounds produced by these strains.The study reveals that the Kalar Kahar lake is a promising source of bioactive actinomycetes. The fermentation, isolation, purification and structure elucidation of the compounds produced by these isolates may yield commercially useful antimicrobial agents.

PP-105

Designing and Development of Synonymous Hepatitis B Surface Antigen Gene for Overexpression in *Kluyveromyces lactis*

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Hepatitis B virus infection is a serious threat to the human health worldwide. It is a crucial public health problem in Pakistan with increased morbidity and mortality. Effective prophylaxis is available against HBV but is not responsive in all cases and despite the availability of vaccine new infections remain common due to mutations. HBV vaccine is not manufactured in Pakistan as is imported so vaccine is expensive and out of reach of an ordinary person. This study contributes toward the development of a local, cost effective vaccine against HBV infection. Hepatitis B surface antigen gene of genotype D and sub type ayw was selected as a vaccine candidate because it is prevalent in Pakistan. Gene sequence was taken from National Center for Biotechnology Information and was modified according to Kluveromyces lactis preferred codons in order to enhance the expression level of protein in heterologous host K. lactis. Relative synonymous codon usage approach was used to enhance the expression. Computational analysis showed that designed gene is a good candidate for vaccine development. Synthetic gene was sub cloned into yeast expression vector pKLAC1 under the control of PLAC4-PBI inducible promoter. Recombinant plasmid was then transformed into Escherichia coli DH5α cells. The cloned HBsAg gene will be expressed in eukaryotic expression system for expression of properly folded protein that can be used as vaccine against hepatitis B virus infection. This cost effective vaccine will be helpful in alleviating the financial burden of low income hepatitis B patients.

PP-106

Study of Lipid Profile in Obese and Cardiovascular Disease Patients and a Common Variant of FTO (Fat mass and Obesity associated) Gene, rs9939609, in Pakistani Population

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Obesity refers to excess of body weight. A World Health Organization (WHO) Consultation described obesity as a chronic disease which is increasing all over the world replacing traditional health concerns. Prevalence of obesity has increased over last decades in Britain, United states, and other countries. Although weight gain and fat storage have been considered as sign of prosperity since long time, criteria have now changed. First clinical reports of obesity date as far back as Graeco-Roman timeshowever, little scientific progress in the field of obesity was made until 20th century. Obesity affects all age groups, children as well as adults. It is considered to be associated with an increased risk of cardiovascular diseases, diabetes, hypertension, physical inactivity, dyslipidemia and others. Two hundred samples from obese individuals and age and sex matched controls were collected after informed consent. 57 individuals were found to have cardiovascular problems. Lipid profile (Total cholesterol, LDL cholesterol, HDL cholesterol, and Triglycerides) was determined for obese as well as cardiovascular patients using commercial kits available (Spectrum Diagnostics). rs9939609 was genotyped using allele specific PCR. Lipid profile was significantly changed in obese persons as compared to population mean (CI 95%, $p<0.05$) while in cardiovascular patients HDL cholesterol values were even lower as compared to other obese individuals. rs9939609 polymorphism was present in homozygous state in three individuals. The frequency of allele A is 0.98 while that of allele T is 0.02, frequency of AA genotype is 0.96, TT genotype is 0.0004, and AT genotype is 0.0392. the study indicates that lipid profile is significantly associated with body weight, it can be a determinant for progression from obesity to cardiovascular problems and polymorphisms may contribute to an elevated body weight in Pakistani population.

PP-107

Evaluation of Antimicrobial Action of *Withania coagulans* Against Various Bacterial Strains of Pyogenic Skin Infection

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Background: Withania Coagulans commonly known as Paneer doda in Pakistan is a traditional medicinal herb, known to be a cure against many diseases such as diabetes, etc.This research focuses the antimicrobial activity of Withania against skin infection

isolates. Method: Aqueous extract of Withania was tested for its antimicrobial activity against various bacterial strains as such as Escherichia coli, Staph.aureus, Pseudomonas Aeruginosa, Bacillus Subtilis, and Protease mirabilis by disc diffusion method and agar well diffusion method. Result: The Withania coagulans aqueous and chloroform extracts showed antibacterial activity against pyogenic skin isolates. In comparable, several antibiotics were tested alongside the isolated organisms. Conclusion: The information demonstrates potential outcome for Withania Coagulans in contrast to five different antibiotics. Moreover, analysis also confirmed that the pyogenic organisms were challenging besides several antibiotics. This study opens a new dimension whereby plant extracts may be employed for antimicrobial treatment.

PP- 108

Elemental Analysis of Plant Derived Smoke Solutions and their Effect on Seed Germination and Plant Growth

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Aerosol smoke and aqueous smoke extract derived from burning vegetation is widely recognized as a germination cue for seed germination and recent reports suggest that smoke treatments can improve seedling vigour also. The present study aimed at determining the effect of active compounds and the possible nutrient elements in different smoke solutions on seed germination and plant growth of maize. Smoke solutions were prepared from aerial parts of 25 different plants. Eight major and minor elements (N, Ca, Mg, K, Na, Fe, Mn and Cu) in samples of smoke solutions were analyzed. Germination and plant growth trials were conducted on maize kernels. The results showed the presence of eight elements in about all the smoke solutions, varying in concentrations. The aqueous smoke extracts were found to contain, N (0.282-6.27%), Ca (2.05-28.8 ppm), Mg (0.301-10.42 ppm), K (0.10-38.5 ppm), Na (0.20-56.1 ppm), Fe (0.005-32.85 ppm), Mn (0.011-0.70 ppm) and Cu (0.014-0.049 ppm). Results from both petri plates and sand culture experiments suggest that most of the smoke solutions significantly ($P<0.05$) improved germination %, vigour index, root length, number of lateral roots, number of leaves, shoot length and root and shoot fresh and dry weights. These stimulatory effects were more effective at higher dilutions. An inhibitory effect of most of the concentrated smoke solutions and few lower dilutions was observed on various growth parameters of maize, possibly due to high concentration of inhibitory compound in smoke solutions. Positive correlation was observed among most of the nutrient elements and the various growth parameters of maize, suggesting the possible role of mineral elements along with active compounds (in smoke solutions) in seed germination and plant growth.

PP-109

Real Time Polymerase Chain Reation (qRT-PCR) Assay Based Molecular Diagnosis of Brucellosis in High Risk Occupations in Pakistan

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The present study was conducted on real-time polymerase chain reactions (qRT-PCR) assay based molecular diagnosis of brucellosis in humans at high risk in the Potohar plateau of north eastern Pakistan. A total of 262 serum samples were collected from persons of different occupational groups: Veterinary personnel, Milkers, Abattoir workers, Livestock farmers and others (Drivers, Security guards, House wives). Data related to gender, age, occupation, contact with animals, brucellosis-related symptoms, consumption of raw milk and geographical region were collected. DNA was extracted from serum samples by High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. DNA purity and concentration was checked using a Nano-Drop ND-1000 UV-Vis spectrophotometer (Nano-Drop Technologies, Wilmington, DE). For the molecular detection of Brucella one genus (bcsp31) and two species specific (IS711) qRT-PCR for B. abortus and B. melitensis were used. The overall seroprevalence was found to be 6.9%. The qRT-PCR showed that all cases were affected by Brucella abortus. Moreover, taking into account one genome equivalent (GE) equals 3.38 fg, the analytical sensitivity of qRT-PCR was 296 GE / µl. This is the first report of human brucellosis related to B. abortus in high risk professionals from Pakistan by the use of molecular methods.

PP-110

Whole Plant Response to Plant Derived Smoke Extracts with Special Reference to Pollen Viability

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Plant-derived smoke aqueous extract is used as a growth regulator for last few decades, for improvement of seed germination and seedling vigor. The present work was designed for the first time to investigate the effect of plant-derived smoke aqueous extract on whole plant response i.e. seed germination, plant growth (root and shoot vigor), pollen viability and yield of Brassica spp. and maize plants and devising specific dilutions of specific plant smoke treatments to enhance the various parameters at various stages of plant growth. Plants were subjected to plant derived smoke solutions via seed priming and foliar spray in fields and pots. Pollen grains at flowering stage were collected and cultured in germination medium suggested by Brewbaker and Kwack to analyze pollen viability. The present results indicate that plant derived smoke extracts enhanced seed germination, plant vigor, pollen viability and plant yields. As plant derived smoke extracts extract have plant growth regulators like compounds so it can be used as a plant growth regulator and fertilizers for enhancing crop productivity.

PP-111

Polymorphism in Exon 6 of CYP11B1 Gene in Sahiwal Cattle

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Sahiwal cattle is considered the best breed among Zebu cattle existing in Indo-Pak region due to its high milk yield, resistant to parasites and heat tolerant traits. Cattles are subjected to certain genetic, environmental and hormonal factors for the regulation of production and reproduction traits. Steroid 11β-hydroxylase (CYP11B1; cytochrome P450, subfamily XI B, polypeptide 1) gene is concerned with the catalysis of 11-deoxycortisol to cortisol and 11- deoxycorticosterone to corticosterone in cattle. Steroid hormones are physiological regulators and cortisol is one of the principal hormones involved in lipogenesis and lipolysis. The CYP11B1 gene, positioned on BTA14q12, influence fluid volume, electrolyte homeostasis, glucose and lipid metabolism. The current study was aimed to identify the single nucleotide polymorphism in coding region of CYP11B1 gene in Sahiwal cattle breed of Pakistan. A total of five polymorphic sites were identified in exon six of CYP11B1 gene through sequencing approach. Remarkable finding of the current study was the incidence of two adjacent nucleotide changes at P1306512 and P1306513 in the order of A→G and C→A. Both of these SNPs constitute a single codon representing the polymorphism from threonine to Aspartic acid (T372D) as the change in amino acid codon from ACC→GAC. That Thr/Asp polymorphism may serve as a powerful genetic tool for the development of DNA markers that can be used for the production and reproduction traits for different local cattle breeds.

PP-112

Interleukin-6 Gene -174G>C Promoter Polymorphism and Risk of Acne vulgaris in Pakistani Population

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Acne vulgaris is a multifactorial disease affecting a majority of adolescents. Follicular hyperkeratosis, seborrhea, Propionibacterium acnes propagation and inflammation are the pathophysiologic factors that influence the acne development. Interleukin (IL)-6 is one of the potent mediators of inflammation implicated in the pathogenesis of acne. A number of polymorphisms recognized in the human IL-6 gene has been involved in increased susceptibility of inflammatory disorders. The aim of this study was to investigate IL-6 -174G>C gene polymorphism in acne and to determine whether there is a relationship between this polymorphism and disease severity. A total of 149 patients (100 females and 49 males) and 104 healthy controls (54 females and 50 males) were enrolled in this association study. Acne vulgaris patients were classified in mild, moderate and severe acne groups depending upon the diseases severity. Polymorphism in IL-6 gene promoter was studied using restriction fragment length polymorphism method. The GG genotype at position -174 in the IL-6 promoter was more frequently observed in acne patients than in controls although not statistically significant. A significant association appeared between the IL-6 -174 G>C genotype and severity of acne vulgaris in male patients (P=0.008). However, IL-6 -174G>C genotype and allele frequencies did not significantly differ between female acne vulgaris patients of different severity and control subjects (P>0.05). In conclusion findings of this preliminary study suggest that IL-6 -174G>C promoter polymorphism may contribute to the severity of acne vulgaris in Pakistani population. Further research is warranted to delineate the association of IL-6 -174G>C polymorphism with acne vulgaris accompanying large sample size.

PP-113

Role of Serum Cholesterol, Triglycerides and VLDL-C Levels in Pathogenesis and Severity of Acne vulgaris in Pakistani Patients

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Acne vulgaris is the most common chronic inflammatory dermatosis of pilosebaceous unit characterized by non-inflammatory comedones and inflammatory papules, pustules,

nodules, and cysts. The aim of this study was to find lipid profile in patients with acne vulgaris and to compare cholesterol, triglycerides and VLDL-C levels in Pakistani patients, grouped on the basis of disease severity, family history, gender and acne types. Prospective study was performed in the 202 patients (male=59, female=143) and 278 healthy controls (male=187, female=91). Serum cholesterol and triglycerides levels were estimated in acne vulgaris controls and patients by end point spectrophotometric method. Student's t-test was used to compare lipid profile results of acne patients with age and sex matched healthy controls. Total cholesterol was significantly higher in both male and female patients when compared with male and female control subjects respectively (P< 0.0001). However, triglycerides and VLDL-C levels were same in both male and female patients and controls (P>0.05). Serum cholesterol level was significantly elevated in severe acne group compared with mild and moderate (Mean ± SD: 183 ± 57.5 vs 169 ± 39.5 and 169 ± 54.6 mg/dL respectively) acne groups (P<0.05) but triglycerides and VLDL-C levels were same in patients with mild, moderate and severe acne vulgaris (P>0.05). High cholesterol level was observed in female patients than male patients (Mean ± SD: 170 ± 45.9 vs 136 ± 55.9 mg/dL) but triglycerides and VLDL-C concentrations were same in both genders. Cholesterol, triglycerides and VLDL-C levels were similar in patient groups with positive and negative family history of acne vulgaris (P>0.05). In papulopustular type of acne vulgaris levels of serum cholesterol, triglycerides and VLDL-C were same as estimated in nodulocystic acne type (Mean ± SD: 180 ± 54.3 vs 164 ± 44.4, 185 ± 50.4 vs 173 ± 50.1, 37 ± 16.3 vs 34 ± 10.0 mg/dL respectively). This study revealed a significant association between serum levels of cholesterol with acne severity. This abnormality must be considered in the acne vulgaris pathogenesis and treatment.

PP-114

Effect of Inoculant Concentration on Safety and Nutritional Quality of Corn Silage

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Fodder crops including maize, grasses, legumes, wheat and Lucerne can be persevered for offseason utilization by ensiling. In many country ensiled forage are highly valued as animal feed. High quality silage involves good microbial fermentation process. A high quality silage production depends not only on type and quality of crop but also type and quantity of microbial inoculum. The present work was designed to evaluate the effect of microbial concentration on safety and nutritional quality of corn silage. Three different inoculant concentrations (2,4,6 gm/ton) were applied and ensiling was done for forty five days. Samples were analyzed at 0, 7th and 45th days of fermentation. Total bacterial and yeasts counts was increased with increase in the concentration of inoculant while

lactobacillus count was highest at 45th day in 4gm/ton inoculation level. All samples at were positive for Listeria monocytogenes, however only two and ten sample were positive for Clostridia sp and by coliform. The different level of inoculants did not have significant difference in nutritional quality of silage. It could be inferred from the study that more precised selection and characterization of microbial strain is need before using them as a commercial inoculant.

PP-115

Relationship of Host Serum Markers with HCV Response to Treatment

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Hepatitis C virus (HCV) is a global public health problem. There are several mechanisms evolved by the HCV that facilitate the persistence of virus and further lead the patient's status as non-responder. However, the precise mechanism underlying viral non-response to treatment is still not clear. Present study was conducted to explore role of patient's serum markers in viral resistance's to therapy. Initially 500 HCV ELISA patients were selected who met the selection criteria. Among these, 451 patients were constantly followed throughout the study period and were divided into two groups on the basis of their treatment response. Group 1 constitutes the 376 patients who became HCV RNA negative in response to therapy while group 2 comprised 75 patients who did not respond to therapy at all and remained HCV RNA positive even till the end of therapy. Patients were diagnosed as non-responders or responders on the basis of viral load determined by Real-Time PCR. Both the groups 1 and 2 were compared with positive and negative control groups. Statistical analysis was performed. The selected study cohorts showed the high ratio of male patients as compared to females with majority of chronic cases. Patients with high baseline viral load showed the low response rate and remain non-responders as compared to the patients with low baseline viral load. Moreover statistical analysis showed that all the serum markers related with liver diseases showed significant disturbance in non-responders as compared to those who become HCV RNA negative in response to therapy. No significant relationship was found between the viral load and serum markers. It can be suggested that besides patient's gender, disease stage and viral load, host serum markers might play important role in HCV response to treatment and can act as predictive factor for therapy response.

PP-116

Characterization of Partially Purified Lac Produced by *Ganoderma lucidum* in Solid State Fermentation

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Lignin, a constitutive material in plants, is the second most abundant natural polymer. Laccase has potential application in the pulp, textile and paper industry. *Ganoderma lucidum*, a white rot basidiomycete was studied for the production and optimization of laccase. Different substrates like wheat straw, rice straw, banana stalk, sugarcane bagasse, corn Stover and corn cobs were used in solid state fermentation (SSF) for the production of laccase by *G. lucidum.* The cultures were incubated at 35 °C for 10 days; samples were harvested after every 24 hours to check enzyme profiles. Maximum enzyme activities were seen on 10th of incubation on wheat straw and so laccase activities were further optimized at 10th day of incubation using wheat straw as a substrate. Maximum production of laccase were obtained when wheat straw at moisture level 60 % and pH 4.5 was incubated with 5ml homogeneous spore suspension of *Ganoderma lucidum* and was incubated for ten days, at 35^{o}C in the presence of glycerol as carbon source and urea as nitrogen source supplement. It was noted that due to the addition of different carbon and nitrogen sources the enzyme production were enhanced. The best combination for Lac at which maximum activity was observed is 5:1 combination i.e glycerol and urea in case of *Ganoderma lucidum.* Results of optimized experiments indicated that the solid-state fermentation parameters indicate moisture contents, carbon and nitrogen sources, carbon and nitrogen ratio, inoculum size, mediators and metal ions has the significant influence on the growth of *Ganoderma lucidum* and laccase production. The enzyme produced by *Ganoderma lucidum* was partially purified by ammonium sulphate precipitation and gel filtration chromatography. The purified enzymes were characterized through kinetic studies by studying the effect of varying pH, temperature, substrate concentrations and activators / inhibitors for its possible applications in industrial processes.

PP-117

Temperature and Climate Affect the Endophytes Community in Grapevine

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Science has just started understanding how the environment drives the composition of microbial communities. Endophytes, as host-associated microbes, respond to

environmental stimuli in a host-mediated fashion. To study how temperature and climate may affect endophytic microbial communities, we studied grapevine-associated microbial populations using a cultivation-independent approach. This experiment used identical potted plants grown in homogeneous conditions at ambient temperature. Each plant was sacrificed at sampling, DNA was extracted from roots (RI) and stems (SH) after surface sterilization and abrasion. Five plants were sampled at time zero (T0) as controls. Three groups of plants were exposed to different temperature (15°C, 25°C, 35°C). After 30 days of exposure, five plants from each group were sampled (T1), the other plants (3 replicates per treatment) were sampled after 90 days (T2) from the start of the experiment. Total DNA was extracted and bacterial 16S was PCR amplified and purified. Multiple reactions were pyrosequenced at once using Roche 454 GS FLX+ Titanium technology, using Multiplex Identifiers during PCR. We adopted a DNA-based approach to the analysis of microbial populations variation as a response to temperature. Potted plants were analyzed to assess how temperature affects microbial endophytic communities in such a unique environment as the endosphere is. These data will be integrated by analyzing the composition of microbial endophytes in open fields by surveying potted plants at different altitudes representing distinct climatic conditions.

PP-118

Genetic Basis and Biochemistry of Cadmium Induced Diabetes Mellitus

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Cadmium is a heavy metal with bluish-white soft appearance, usually found in zinc ore. There are many sources of cadmium exposure e.g. air, water, soil, different ores and plants etc. Cadmium is highly toxic causing many health disorders due to its chemical similarity with micronutrient zinc. One of these disorders is Diabetics. Recent epidemiological studies suggest a positive association between exposure to the environmental pollutant cadmium (Cd) and the incidence and severity of diabetes. In this review, the literature suggests a relationship between Cd exposure, elevated blood glucose levels, and the development of diabetes. In addition human and animal studies indicate that Cd potentiates or exacerbates diabetic nephropathy. Also various possible cellular mechanisms by which Cd may alter blood glucose levels. In addition, some novel findings from our own laboratories showing that Cd elevates fasting blood glucose levels in an animal model of sub-chronic Cd exposure before overt signs of renal dysfunction are evident. These studies also show that Cd reduces insulin levels and has direct cytotoxic effects on the pancreas. Together, these findings indicate that Cd may be a factor in the development of some types of diabetes and they raise the possibility that Cd and diabetes-related hyperglycemia may act synergistically to damage the kidney.

PP-119

Biochemistry of Cadmium Induced Cancer and How Bioinformatics Help in Cancer Studies

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Little is known about the etiology of Cancer, which is an important cause of cancer mortality in developed countries. In the following we have mainly discussed Cancer that is induced by cadmium. We hypothesize that exposure to cadmium is a cause of cancer. Cadmium ia a nonessential metal that accumulates in the human bodies. It is a naturally occurring element in earth's crust, which is chemically similar to two other stable elements, zinc and mercury, of group 12. Cadmium can enter the environment in several ways. Food and cigarette are the largest known potential sources of cadmium. Our meta-analysis of cohorts with high exposure to cadmium is also consistent with an increased risk of cancer. Thus cadmium is a plausible carcinogen. The cadmium hypothesis provides a coherent explanation for much of the descriptive epidemiology of cancer and suggests new avenues for analytical research. Bioinformatics helps to identify the cancerous gene. Microarray technology, Data Bases for results storage and analysis, Gene Profiling and different other computational tools help in cancer study and by using these techniques we can enhance the efficiency of our work.

PP-120

Clinico-Molecular Diagnosis of Bovine Mycoplasmosis and its Control in Karachi, Pakistan

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Mycoplasmosis is a most serious and economically most costly disease of cattle and buffaloes due to its high morbidity and mortality. Bovine respiratory disease (BRD) is a typical disease of buffalo and cattle caused by Mycoplasma bovis (M. bovis) and some other species of Mycoplasma that may also be involved, such as M. bovigenetalium and M. arginini. These species are also important contributors to arthritis, mastitis and genital disorders etc. This type of infections or disorders most of the time persist due to mismanagement and unhygienic condition of the farm. Therefore, 112 animals were examined clinically for respiratory signs, whereas 49 were observed in respiratory distress (nasal discharge, coughing, sneezing and dyspnea). On the other hand randomly 87 lung samples (slaughtered animals) were studied at abattoir for lesions and visually 51 (58.6%) samples were found pneumonic. Of the 51, 34 (66.6%) samples were found culture

positive. These cultures were confirmed by PCR and 25/34 (73%) isolates were found as M. bovis. However, all the isolates were found Mycoplasma using universal Mycoplasma primer. MIC and MBC of tylosin (tylocon 20%) were performed against the isolates of M. bovis which were recorded as 10µg/ml and 190µg/ml, respectively. The other antibiotics were also tested by disc diffusion method; antibiotics as tetracycline (37mm), doxycycline (36mm), ciprofloxacine (30mm), oxytetracycline (30mm), lincomycin (25mm) and norfloxacin (20mm) were found efective against Mycoplasma isolates. Antibiotic therapy such as tylosin, tetracycline, doxycycline, ciprofloxacine and oxytetracycline are recommended for the treatment of Mycoplasmosis.

PP-121

Inflammatory Cytokine Gene Polymorphism in Coronary Artey Disease Patients

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The aim of this study is to analyze the association of TNF-α -1031 T > C polymorphism in the promoter region of TNF-α gene with CAD in a Pakistani population. Design and Methods: For this study angiographically proven CAD patients (n = 80) and healthy control subjects (n = 80) were recruited. Methodology includes DNA extraction of blood samples and genotyping was performed by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) analysis. The data analysis was done for statistical significance using student's t-test and chi-square test. Results: Among baseline characteristics hypertension (P = 0.01) and overweight (P = 0.01) were more prevalent in patients as compared to control group. Biochemical parameters including HDL-C (P = 0.01) and LDL-C (P = 0.03) were statistically significant among both groups. The mutant (C) allele polymorphism appears not to be associated with CAD (OR = 0.85; 95% 0.51 - 1.40; P value = 0.61). Conclusions: Our study concludes that there is no association of TNF-α -1031 T > C polymorphism with CAD. Considering the role of this polymorphism in the pathogenesis of inflammatory disorders, this study should be extrapolated to other populations with a large sample size to confirm our results.

PP-122

MAPS Data Base: Medicinal Plants Activities, Phytochemical and Structural Database

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Drug development from natural sources (medicinal plants) is an important and fast developing area due to the development of new synthetic molecules limitations based on active components or pure chemicals principles. MAPS database is user friendly information resource including the data of more than 500 medicinal plants. This database

includes information about phytochemical constituents and their 2D and 3D structures, percentage of chemical constituents, all activities possessed by medicinal plant (i.e antimicrobial, antidiabetic, anti-insecticidal, antioxidant, wound healing) with the test organism/chemical reported with literature. For the development of this information resource, literature surveys were conducted in different journals, PubChem, PDB, PMC and Pubmed, allowing the creation of a publication data in a library to provide primary information source with each user query. This database can be queried via plant name, chemical constituent, test organism/chemical (i.e bacteria, virus, fungi) and activities. WAMP server, mySQL, PHP, HTML and CSS were used for the development and accessibility of this database. This database provides useful information about not only including in different databases/literature but also includes Pakistan's medicinal plants missed in them and its medical field application helping researchers working on drug discovery from natural resources. It facilitates user to advance search (selecting more than one plant/activity/test organism/chemical), download results/structures, store their results to use whenever they want, submission, as well as suggestion.

PP-123

Standardization of Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) Diagnostic Test for Rapid Detection of Foot & Mouth Disease Virus

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Foot and Mouth Disease Virus (FMD) is an RNA virus, member of Picornaviridae. It has seven serotypes with no cross protection among these.FMD is responsible for heavy economic losses and drastic effect. So rapid and accurate diagnosis of FMD is uttermost need of this time.Several molecular techniques are developed for rapid detection of FMD but these are expensive and time consuming. These methods are Elisa, cytopathic effects in cell culture, reverse Transcriptase (RT-PCR) and real time PCR. All these need specific high quality equipments in lab. Whereas, Lamp PCR is simple and accurate test for the rapid diagnosis of FMD. This study has been done initially with FMD known serotypes (O,A,Asia1) already in use at Animal Health.Viral RNAs were extracted using RNeasyMinikit (Qiagen) according toinstruction manual.After extraction RNAs were eluted in 60 ul elution buffer and stored at -70C. Almost 100 sequences of 3D gene of FMD were analysed using a Clustal V method (DNASTAR, Madison, WI, USA). A highly conserved region of 3 D gene were chosen and set of four common primers have been designed .HydroxyNephthiol blue used as colour indicator in this test. Each strain of FMD virus was serially diluted to -10 dilution in order to check the efficacy of test. Reaction was completed in 1 hr and 10 min at temperatures 60^0 C. It was also tested in hot water bath on same temperature. Results were

same in both techniques. Positive reactions showed sky blue while negative reactions remain violet. Results showed that LAMP was more sensitive for FMD strain O (positive reaction upto -4 dilution)than others strains i.e, A and Asia -1 (positive reaction upto -3 dilutions). Further studies to develop a field test which does not require RNA extraction is in progress.

PP-124

Phylogeny of Pakistani Cattle Breeds using Mitochondrial Cytochrome B Gene

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According to zooarchaeological data the Near East and the Indus Valley was the center of domestication and diffusion of modern day cattle. To get more knowledge and insight of origin and genetic diversity of native cattle breeds (Bos indicus) in Pakistan, partial sequencing of the mitochondrial gene cytochrome b (339 bp) was done in 136 individuals from 10 different breeds. The analysis of the cytb gene showed high conservation in all the Pakistani cattle, as expected, with only 26 individuals showing nucleotide changes. Only 5 point mutations were present in multiple individuals (SNPs), but one was specific for indicine cattle. Two Lohani and 5 Nari Master cattle showed nucleotide changes specific to taurine cattle. Of the changes found, only three produce amino acid change in the protein sequence. The UPGMA tree showed a clear differentiation between taurine and indicine cattle, except for those Pakistani cattle showing mitochondrial taurine sequences because they are mixed-bred. The within breed estimates of divergence were very low in all breeds except for Nari Master (mixed-bred). The estimates of divergence among breeds were also low for most breed pairs, except for Nari Master and Dhanni. The overall divergence within the B. indicus or within B. taurus were also very low (0.002 and 0.003, respectively) but the difference between B. indicus and B. taurus was significantly higher (0.014). Keywords: Pakistani Cattle, Cytochrome b, Genetic diversity, Phylogenetics

PP-125

Role of Kisspeptin-10 in the Proliferation and Differentiation of r366.4 Cells

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Kisspeptins is a product of metastasis suppressor gene (KISS-1) and a ligand for GPR54 (G protein coupled receptor). Earlier studies suggest that Kisspeptin plays a major role as metastasis suppressor in humans. Kisspeptin-GPR54 signaling has previously emerged as a potent regulator of the reproductive axis, elicitor of GnRH and gonadotropin secretion. It has also been studied that kisspeptin treatment can cause dendritic extensions in GnRH in the brain slices'. Recently, the effect of different doses of kisspeptin has been observed on different cell line; however, the role of kisspeptin-GPR54 in cell proliferation process is not clearly understood until now. The stem cells and stem cell based therapy carries great potential in neurodegenerative diseases. Therefore this study was designed to determine the effect of the human kisspeptin-10 on r366.4 (Rhesus monkey embryonic stem cells) cells. Four different doses of kisspeptin-10 were selected, i.e., 100nM, 10nM, 1nM, 0.1nM. On day 12 (the early rosettes stage), the cells were treated with Kisspeptin-10 for 24, 48 and 72 hrs. Afterwards proliferation rate was analyzed by flow cytometry and cell count method. The number of rosettes was also counted manually under the light microscope. The change in the morphology was critically observed. Kisspeptin-10 treatment decreased the number of cells (anti-proliferative effect) and rosettes both dose and time dependently, without affecting the viability and apoptosis of the cells. From this preliminary study we can conclude that kisspeptin is inhibiting the proliferation rate of the r366.4 cells and significant decrease was observed at the higher dose from which we can assume that these doses of kisspeptin might enhance the endogenous GPR54 present in the cells. The increase in the expression of GPR54 resulted in the increased response to the drug (anti-proliferative effect). As the proliferation increases, the number of the neuronal rosettes decreases which are the hall mark for the differentiation. Another finding is the change in the shape and size of the cell bodies of the growing neuronal stem cells.

PP-126

The Influence of Activated Charcoal in Establishment of Regeneration Protocol for Lentil (*Lens culinaris* Medik)

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The study is aimed to establish an efficient regeneration protocol for recalcitrant legume, lentil, by optimizing various factors with use of activated charcoal. Two lentil varieties, three explants, eight shooting media combination and 10 rooting media combination were used in this study. Masoor-2002, a variety from microsperma group showed 100% shoot development on media containing 0.1 mg/L GA3, 5.5 mg/L Tyrosine, 0.25 mg/L Kinetin, 1.0 mg/L BAP using cotyledonary node as explant. Addition of charcoal in shooting media resulted in healthier plants but number of shoots was reduced. Rooting of developed shoots was done on medium with or without charcoal. Healthy and higher number of roots was observed on media containing 4 mg/L IAA and 2 mg/L charcoal. Regeneration was obtained from nodal fragments on shooting media supplemented with 1.0 mg/L BAP and rooting media with 1 mg/L IAA. Plants were established better in soil less medium containing perlite, vermiculite and peat moss in 1:1:1 ratio as compared to garden soil and sandy soil.

PP-127

ELISA Based Serosurveillance of Bovine Fascioliasis in District Sargodha

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Serological and coprological survey of bovines of district Sargodha was carried out. A total of 5580 fecal samples and 600 blood samples were collected from all six tehsils of district Sargodha. Sedimentation-floatation technique was adopted to identify Fasciola eggs in feces. Sera were screened for presence of antifasciola antibodies by indigenous DRG kit. The highest prevalence was found during month of December in both serological and coprological examination. Higher prevalence was found in Bhalwal, Sahiwal and Shahpur tehsils as compared to Sargodha, Kot-Momin and Silanwali tehsils. During coprological survey of bovines of district Sargodha, Fasciola eggs were identified in 1962 animals out of 5580 cattle and buffaloes. Significantly higher prevalence (χ^2=5.8399; P-value=0.0157; OR=0.563) was found in buffalo population as compared to cattle. Prevalence of F. gigantica was significantly higher (χ^2=70.6325; P-value= 0.0001) between the two species. DRG kit ELISA both have detected antifasciola antibodies in higher percentage in buffaloes (49.16% respectively) as compared to coprological examination (True prevalence= 39.33%). Similar results were recorded in cattle. Higher seroprevalence was determined as 39.36% as compared to 30.67% in coprological examination. Risk of fascioliasis was found to be negatively associated (OR=1.181; χ^2=105.6757; P-value <0.0001) with age categories being highest prevalence of fascioliasis in >2-4 years age group and then decreasing with advancement of age. Sex was found non-significantly associated with disease. Among management practices, higher prevalence was found in grazing group (χ^2=61.3443; P-value

<0.0001), pond watered and river watered group (χ^2=89.7096; P-value <0.0001) as compared to stall feeding and tap watered group.

PP-128

Distinctive Firing Properties of Thalamic Neurons in Novel Environment

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Introduction: Thalamus is a vital brain area, involved in sensory perception and regulation of motor function. It plays crucial role in multiple physiological processes (learning and attention) and pathophysiology of brain disorders (epilepsy etc.). Thalamus is divided into many small sub-thalamic areas. Thalamic cells fire action potential in either tonic or burst firing mode. Aim: The aim of this study was to understand the role of mediodorsal and non-mediodorsal thalamic neurons in novel environment. Methods and Results: To understand the distinctive role of mediodorsal thalamic cells (MDCs) and non-mediodorsal thalamic cells (non-MDCs), these cells were recorded in mice (subjected to novel environment), using tetrodes technology. MDCs showed increased bust firing events/minute as opposed to the non-MDCs (0.25 ± 0.10 vs 1.53 ± 0.49 p = 0.02) after 50 minutes of novel environment exploration. On the other hand, compared to the initial baseline, non-MDCs showed larger decrease in percentage tonic firing frequency as opposed to the MDCs (39.80 ± 2.3 vs 61.44 ± 15.19), after 50 minutes of exploration. Conclusions: These results show a regulatory role of MDCs and non-MDCs in novel environment and demonstrate multifaceted functions of different thalamic cells. Further studies can help to understand the pathogenic mechanism of attention-deficit and other thalamic disorders and can also help in identifying the diagnostic features for such disorders.

PP-129

Epigenetic Analysis of Embryonic Stem Cells Specific Genes in Blood of Normal and Leukemic Patients

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Epigenetic changes is one of the many causes of cancer. The main epigenetic change in cancer cells is the alteration of methylation pattern of CG Island in the promoters of tumor suppressive and oncogenes. An exciting and emerging theme is the unraveling of the expression of embryonic stem cells specific genes in the adult cells and their roles in the progression of cancer. The plueripotentcy of the embryonic stem cells is maintained by the expression of specific transcription factors such as OCT4, Sox2, Nanong, c-myc and Klf4.

Compared to somatic cell cycle, the expression of these transcription factors alter the regulation of cell cycle in embryonic stem cells resulting in the shorter G1phase in embryonic stem cells. Our research interests lie in assessing the alteration in the methylation of CG in the promoter of OCT4 and SOX2 genes in the DNA of (acute lyphoblasticLeukaemia) patients. We assessed the alteration of methylation of four CG dinucleotides present in CCGG sequences in the upstream regulatory region of OCT4 and SOX2 by restriction digestion of CCGG using MSPI and HPaII enzymes followed by PCR to amplify the region flanking the CCGG. This simple yet effective methodology was used to assess the alteration of methylation in the three CG dinucleotides in the SOX2 and OCT4 genes in the DNA extracted from 60 control and 50 ALL patients. CCGG sequence at 3 loci was selected in the 2.7 Kb upstream regulatory region of OCT4 gene and at one locus, 370bp upstream from translation start site of SOX2 gene. The PCR bands were observed in the control while they were absent in the DNA of ALL patients indicating the alteration of methylation at these four CGs. Our results demonstrate that the specific CG in the CCGG inside the promoter of OCT4 and SOX2 undergoes demthylation in the ALL patients compared to normal. However, further research is needed to confirm our preliminary findings and the roles of OCT4 and SOX2 in the progression of ALL.

PP-130

Prevalence and Trends of HBsAg, Anti-HCV and Anti-HIV among Blood Donors at Pakistan Institute of Medical Sciences, Islamabad, 2005-2012

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Pakistan is a developing country of 190 million people with increased burden of infectious diseases. Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Human Immunodeficiency virus (HIV) are the most important agents responsible for transfusion transmitted viral infections. Blood donors are considered as the healthiest population of a community and screening of HBV, HCV and HIV in blood donors will reflect the true prevalence of these infections in a population. A retrospective analysis of blood bank datafor last seven years (from July 2006 to June 2012) was carried out.All blood donors were screened for HBsAg, anti-HCVand Anti-HIV by enzyme linked Immunoassay technique (ELISA) using protocols supplied with respective kits.A total of 136, 960 blood donations were processed during this period. The proportion of voluntary donations (n=2,362; 1.72%) was significantly lower than replacement blood (n=134,598; 98.28%) (P<0.001). The overall prevalence of HBsAg, Anti-HCV and Anti-HIV was found to be 2.11%, 3.27% and 0.01% respectively. HBV/HCV co-infection was reported in 117 cases. For analysis of change in trend, the data

was segregated into group A comprising of cases from July 2006 to 2009(n=71,896) and group B (n=65,064)spanning from 2010 till June 2012. Analysis revealed a significant increase in prevalence of HBsAg, HCV and HIV respectively. The prevalence of HBsAg rose from 1.856% (1339 out of 71,896) in group A to 2.38% (1,551 out of 65,064) in group B (P<0.001). Similarly significant difference was observed between the two groups for prevalence of HCV and HIV (3.14% vs 3.41% ; P=0.005 for HCV and 0.0069% vs 0.02% ; P<0.001 for HIV) respectively. The incidence of HBV/HCV remained consistent (56 vs 61 cases) during this period. An increased incidence rate of TTI call for rigorous measures including procedural improvements and more stringent donor selection criteria with emphasis on non-remunerated voluntary blood donations.

PP-131

Evaluation of HIV/AIDS Diagnostics Kits and Formulation of a Testing Strategy for Pakistan

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Rapid diagnosis of HIV/AIDS enables the development of prevention and treatment programmes but accurate and cost effective testing strategies should be used for testing of HIV/AIDS from a large population. This study evaluated the performance and effectiveness of three assays for diagnosis of HIV in comparison with Western blot and to formulate an alternative cost-effective confirmatory approach for HIV diagnosis that is suitable for use in Pakistan. The study was conducted at the National HIV/STI Referral Laboratory, National AIDS Control Programme. 472 specimens (serum) were evaluated over a period of six months. Two commercially available HIV testing kits (Capillus HIV-1/HIV-2, SD Bioline HIV-½ 3.0) and one commercially available ELISA kit (Vironostika HIV Uni-Form II Ag/Ab) were used to detect HIV and results were compared with Western blot (Genetic Systems HIV-1). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of all HIV assays were assessed against WB. 280/472 (59.3%) of the samples were positive for antibodies against purified HIV-1 viral proteins. The sensitivity of SD Bioline and Vironostika HIV Uni-Form II ELISA was 100% (95% CI; 98–100) while that of anti-HIV CapillusTM kit was 94.6% (95% CI; 91–96.8). The specificity of the Vironostika HIV Uni-Form II ELISA and anti-HIV CapillusTM kit was 100% (95% CI; 97–100) while specificity of SD Bioline was 98.4% (95% CI; 95–99). PPV was 100% (95% CI; 98–100%) for the anti-HIV CapillusTM kit and Vironostika HIV Uni-Form II ELISA and 98.9% (95% CI; 96–99%) for SD Bioline HIV-1/2 3.0. NPV for SD Bioline HIV-1/2 3.0 and Vironostika HIV Uni-Form II ELISA was 100% (95% CI; 98–100%) and 92.7% for anti-HIV CapillusTM kit (95% CI;

88–96%). The sensitivity and specificity of all three kits were satisfactory compared to western blot and could form the basis of a testing strategy for effective diagnosis of HIV/AIDS in Pakistani population. However, there are many challenges associated with executing the proposed algorithm, ranging from structural (policy, law) to operational (staff training, developing quality assurance protocols) and technical. It is recommended that the government should assure implementation of guidelines and provide resources for the validation of national testing strategies for HIV diagnosis on a regular basis over time.

PP-132

Leptin Replacement Therapy: A Treatment of Childhood Obesity

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Leptin is a hormone that is formed in the white fat tissues in humans and helps in regulation of the appetite. Leptin regulates food intake and energy expenditure by acting on the two populations of arcuate neurons in hypothalamus, these are POMC/CART neurons that are activated by action of leptin; and AgRP/NPY neurons that are inhibited by leptin. Congenital leptin deficiency is a rare human genetic condition clinically characterized by hyperphagia and acute weight gain usually during the first postnatal year. This condition arises when the leptin cannot be formed or secreted in blood to function in form of normal protein. Till now six pathogenic mutations have been reported in the leptin gene, that render the protein inactive or nonfunctional. Childhood obesity due to leptin deficiency is more common in Pakistani population compared to rest of the world as most of children presented with congenital leptin deficiency are from Pakistani origin (75 % 21of 28 total reported cases). The condition of leptin deficiency is resolvable with the exogenous leptin replacement therapy. Trials of human recombinant leptin replacement are under process and so far yielded successful results in reducing weight, controlling appetite and enhancing immune and endocrine function. Nine children diagnosed with leptin deficiency in Pakistan have been selected to get this trial treatment in Germany.

PP-133

Exogenous Application of Salicylic Acid for Improving Growth and Yield of Sweet Pepper

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Salicylic acid (SA) is an important signal involved in the activation of defence responses against abiotic and biotic stress. A study was conducted with aim to induce the systemic acquired resistance for improving plant growth and yield in sweet pepper (Capsicum annuum) by using different concentrations of SA. SA was sprayed at three different stages. Different SA treatments succeeded to increase pepper yield. The results showed also that 0.1 mM and 0 .3 mM of SA were the most effective concentrations. The results showed that SA treatments induced a significant increase in proline and ascorbic acid content. In addition, different SA treatments succeeded to increase ascorbate peroxidase activity, while, decrease H2O2 level, lipid peroxidation and SOD activity. Peroxidase and catalse activity in C. annuum decreased under SA treatment. Exogenous application of 0.1 mM SA increased net photosynthetic rate, stomatal conductance, carboxylation efficiency and yielding parameters over control treatment. Increase in growth and photosynthetic capacity due to exogenously applied SA may have been due to SA-induced increase in activity of peroxidase.

PP-134

Molecular Status of β-Thelassemia in the Population of Central Punjab, Pakistan

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Beta-Thalassemia is recurrent monogenic disorders in the Southeast Asia and having major severe phenotype which necessitates enduring blood transfusions and bone marrow transplant is the only restorative option available. Alertness of the cultural and geographic origin would make possible molecular investigation to be modified and eminent occurrence in convinced population. The investigation of 168 unrelated beta-thalassemia chromosomes consisting of 84 transfusion dependent children from central Punjab including Lahore, Faisalabad and Sargodha regions of Pakistan were characterized for beta-thalassemia by using Monoplex ARMS-PCR, Multiplex ARMS-PCR, restriction endonuclease analysis and allele specific oligonucleotide (ASO) hybridization. The thirteen beta-thalassemia mutations were identified from 98.21 % (165/168) of the chromosomes. Our results indicate that three most common mutations IVS-I-5 (G-C), FSC-8/9 (+G) and CD41/42(-CTTT) 66.07 % accounted for the beta-thalassemia alleles of central Punjab of Pakistan. Other minor mutations IVS-I-I (G-T), IVSII-I(G-A), 619 bp del, IVS-II-848 (C-A), CD15 (G-A), CD16 (-C), IVSI-I (G-A), CD 30 (G-C), CD 26 (G-A) and Cap+I (A-C) are contributed only 32.14 %. These conclusions have imperative insinuations for avoidance of beta-thalassemia through genetic counseling and pre-natal verdict in central Punjab of Pakistan.

PP-135

Ectopic Expression of the Transcription Factor AtDREB1A Confers Increased Tolerance to Drought Stress in Tobacco (*Nicotiana tabacum L*)

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Drought is a major limiting factor for plant growth and crop production. In an attempt to develop drought tolerant tobacco, an expression cassette containing the Arabidopsis DREB1A cDNA under the Figwort Mosaic Virus promoter (FMV) was transformed into tobacco via Agrobacterium mediated transformation. FMV is a strong and constitutive promoter that can be used for enhancing expression of AtDREB1A gene in tobacco. Putative transgenic T0 plants were confirmed by PCR and copy number determined by Southern blot hybridization. RT-PCR confirmed the expression of gene in transgenic plants. The selected transgenic plants were further analyzed for drought stress tolerance at T1 generation. Seed germination results showed that transgenic seeds were able to germinate on 20% PEG and 300mM mannitol while wild type seeds failed to germinate. Different physiological tests demonstrated enhanced tolerance to drought stress in transgenic plants than their wild type counterparts. Transgenic plants showed enhanced drought tolerance and produced more seeds than control plants when water was withheld for 10 days. The present investigation clearly showed that overexpression of the AtDREB1A gene under FMV promoter enhances drought tolerance in transgenic tobacco and offers applications in developing drought tolerant crops.

PP-136

Comparative Studies of Fibrinolytic Genes and their Phylogenetic Studies Isolated from Local Bacillus Specie

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Bacterial proteases especially fibrinolytic enzymes are one of the potential enzymes involved in the cure of different cardiovascular disorders by digesting clots and for the treatment of cancers also. Bacterial species adopt these enzymes in order to penetrate the clots thus playing a vital role in its pathogenecity. Commercial and medical importance of these enzymes is increasing every day. A number of these fibrinolytic enzymes are also important from research point of view. The present project was therefore completed isolating two fibrinolytic genes BKII and qk02 from local isolate of Bacillus. The bacterial cells were grown in doubos salts media at 40oC and pH 7.2. The extracted genomic DNA was used to isolate the two genes. Polymerase chain reaction products of each were of 1380 and 1090 base pairs. The two genes were cloned in T/A cloning vector and the samples were sequenced. The sequences were submitted in the genebank and were further used for sequence analysis. The two genes were compared and analysed using BLAST, justbio.com, clustalW, mega4.0 and different types of bioinformatic tools. Comparison was done with their native genes as well as with each other for the similarities the genes share as a part of their common function. Furthermore the genes were compared with other fibrinolytic enzymes also to check the genetic diversity present in the fibrinolytic genes. Phylogenetic analysis was also done.

PP-137

Screening of Microalbuminuria and its Associated Risk Factors in Type-II Diabetics; A Cross-Sectional Analytical Study

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Background: Diabetic nephropathy is a common consequence of long standing diabetes mellitus. The presence of trace amounts of albumin in urine specimen is an early sign of kidney dysfunction and it can be evaluated by simple qualitative screening of microalbuminuria. Objective: Present study aims to screen microalbuminuria in known type II diabetics and its association with plasma glucose level. Methods: This cross sectional analytical study was carried out in PMRC Research Centre Lahore. A total of 130 type II diabetic subject of both genders were selected exclusive of the patients with hematuria, pyuria and urinary tract infection. Information regarding necessary anthropometric, clinical and biochemical parameters including micro and macro albuminuria were collected on a well structured questionnaire. Data was analyzed by using SPSS-20. Results: The mean age of the study subjects was 51.75±10.34 years. Overall prevalence of microalbuminuria was 40.8%. History of co-morbidity showed that 81.5% of the subjects were hypertensive. There is no significant association was seen with microalbuminuria when subjects were distributed according to their BMI. Gender was not significantly associated with microalbuminuria. The prevalence of microalbuminuria positive was 40.0% in overweights whereas in normal weights it was 44.0%. Overall

17(13.1%) had elevated serum urea/creatinine level, among them 7 (41.2%) had microalbuminuria while other 10(58.8%) had macroalbuminuira. Correlation analysis showed that irrespective of presence or absence of hypertension, plasma glucose level seems to be correlated with microalbuminuria (P-Value =0.02) while age, BMI, Waist, duration of Diabetes were not statistically correlated with microalbumin at P<0.05. Conclusion: Elevated plasma glucose level is the major risk factor for the excretion of albumin protein in urine specimens of type II diabetes patients. Policy Message: The high prevalence of microalbuminuria in diabetic subjects increased the risk of nephropathy with time. Screening of microalbuminuria is helpful in early detection of diabetic nephropathy.

PP-138

Identification of Flora of GCU Botanic Garden through DNA Barcodes

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One hundred plant species of GCU Botanic Garden were analysed for their DNA Barcoding using rbcl gene marker by automated DNA Extraction on Beckman/ Coulter Biomek FX (U.Guelph) for the purpose of reconfirming the identification of these plant species at the molecular level and to build up their DNA library. The plant species analysed for their DNA Barcoding were already identified on the basis of their morphological characters described in Flora of Pakistan. The identification was reaffirmed on the basis of the DNA sequences. Moreover, the phylogenetic relationship between the different taxonomic taxa of these plant species was worked out and in most of the cases, the phylogenetic relationship derived on the basis of classical or morphological characters agreed with the results obtained in the present study, i.e. DNA Barcoding, except in few cases, where it was suggested that their DNA Barcoding may either be repeated or should be carried out using other available genes in DNA Barcoding such as mat. K and ITS region of all the plant samples, before making any conclusion.

PP-139

Prevalence of ESBL (Extended Spectrum β Lactamase) Producing Enterobacteriaceae in a Private Hospital, Islamabad

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Extended spectrum β-lactamase (ESBL) producing organisms are a great challenge for clinicians now a day. They secrete β-lactamase enzyme that inhibits the action of certain β-lactam containing drugs like Penicillins, Cephalosporins and Monobactams. Antibiotic

resistant genes transfer between organisms to organisms by plasmids. Antibiotic sensitivity pattern was detected by disc diffusion method on Meuller Hinton Agar. In the year 2012, total 3851 Enterobacteriaceae isolates were reported from Kulsum International Hospital. 2707 (70.29%) were extended spectrum β-lactamase producing organism.From ESBL population,1330 (49.13%) were E. coli, 702 (25.93%) were Pseudomonas, 542 (20.02%) were Klebsiella pneumoniae, 112 (4.14%) were Proteus and 21 (0.78%) were Enterobacter isolates. Many ESBL-producing organisms also express AmpCβ-lactamases which are co-resistant with cefoxitin. For ESBLs synergistic drugs are used in which β-lactamase inhibitors are used such as Clavulanic acid and Augmentin. But resistance against synergistic drugs is also increasing day by day. Carbapenems are the drug of choiceforβ-lactamase producing organisms. But plasmid assosiated carbapenem-resistant bacterial species emerged such as Stenotrophomonassp. Or Pseudomonassp. This retrospective study showed a high ratio of ESBL-producing bacteria in a tertiary care hospital. It can subsequently lead us to the cause of high nosocomial infection index in the hospital since ESBL-producing organisms are one of the causes of hospital acquired infections.

PP-140

Response of Barley to Various Levels of Nitrogen Fertilizer

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Field trials entitled as “Response of barley to various levels of nitrogen fertilizer “were conducted at Cereal Crops Research Institute, Pirsabak Nowshera during 2011-2013. Experiment was laid out in RCB design with three replications. Four levels of nitrogen fertilizers 60, 80,100 and 120 (kg N ha-1) were used. Barley variety Arbab Ayoub Jan was planted on 12th of November. The experiment was repeated with same procedure during 2012-13. Increase in nitrogen level was positively correlated to days to heading, days to physiological maturity and plant height at maturity. Biological yield, grain yield, grain filling duration, number of grain per spike and thousand grain weight were increased with increase in nitrogen level from 60 to 100 (kg N ha-1) but were negatively affected when nitrogen was increased from 100 to 120 (kg ha-1). Minimum days to heading (118) were observed for 60 kg N ha-1 while more days to heading (126) were recorded for 120 kg N ha-1. Less days to physiological maturity (158) were taken at lower dose of nitrogen (60 kg ha-1) while more days (166) were noted when higher level of nitrogen (120 kg ha-1) was applied. Plant height was increased by 16 % (80.3 to 93.4 cm) when nitrogen was increased from 60 to 120 kg ha-1. Biological yield was increased (10490 to 14528 kg ha-1) with increase in nitrogen (60 to 100 kg N ha-1) but again decreased (12473 kg ha-1) when nitrogen was applied at the rate of 120 kg ha-1. Increase (40 to 43 days) in grain filling

duration was recorded with increase in nitrogen (60 to 100 kg ha-1) but again decreased (39 days) when higher level of nitrogen (120 kg ha-1) was applied. In case of grain per spike and thousand grain weight, same trend was observed and these increased (43.17 to 46.83 and 41.85 to 45.50 respectively) with increase in nitrogen (60 to 100 kg ha-1) and then decreased (44.83 and 43.55 respectively) at higher level of nitrogen (120 kg ha-1). Grain yield was improved by 28% (3710 to 4758 kg ha-1) with increase in nitrogen from 60 to 100 (kg ha-1) but more increase in nitrogen (120 kg ha-1) resulted in yield decrease (4387 kg ha-1). Based on the results of this work, it is concluded that 100 kg N ha-1 is recommended for better performance of grain barley in the conditions similar to Peshawar valley.

PP-141

Sequence Analysis of BMP15 Gene in Patients of Premature Ovarian Failure

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Introduction: Premature ovarian failure is a primary ovarian defect characterized by premature depletion of ovarian follicles below the age of 40 years. Patients present with primary and secondary amenorrhea with altered levels of serum FSH, LH and Estradiol. Background and objective: BMP15 Gene has exclusive expression in the ovarian follicles from the third month of fetal life. Various studies on animal model have revealed that variations in bone morphogenic protein 15 (BMP15) may be involved in the pathogenesis of premature ovarian failure (POF. Various previous studies have suggested that BMP15 variations may predispose to POF and contribute in association with other alterations to generate the ovarian defect. Because of its crucial role in folliculogenesis, this study was designed to analyze BMP 15 gene in POF patients from local population. Subject and methods: For this study blood samples (n= 42) were collected from patients of Premature ovarian failure presenting in the outpatient departments of various tertiary care hospital in Lahore. After DNA extraction from blood, the 2 coding exons of the gene were amplified using four primer sets. Direct DNA sequencing was carried out of amplified PCR products. Results: This study revealed a total of 5 sequence variants in the coding region of the BMP15 gene i.e. c.-9C/G (rs3810682), A/G (rs41308602), A/C (rs73488037), G/A (rs104894767), TCT ins Leu. These variations respectively were identified in 38%, 4.7%, 21.4%, 4.7% and 19.04% POF patients. All these variations have already been associated with POF by other laboratories worldwide. These results are in accordance with the studies carried out on other populations which show that the BMP15 gene variants are highly prevalent in patients of POF and can be associated with the etiology of ovarian failure.

PP-142

Biological Evaluation of Extracts and Bio-nanoparticles of selected medicinal Plants of Pakistan

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Medicinal plants has long been used as source of therapeutic compounds for variour alillments. In this study, aqueous and M/C extracts of 61 medicinal plants of Pakistan were evaluated for their antioxidant properties, total phenolic content and total flavanoid content. The total antioxidant capacity was estimated by DPPH free radical scavenging, reducing power and phosphomolybdenum assays. Total phenolics were measured by using Folin–Ciocalteau reagent and total flvanoids were determined by aluminum chloride colorimetric method . The results showed that M/C extracts contain high antioxidant activities compared to aqueous extracts. The reducing power across 122 samples varied from 380 to 59.76 mg ascorbic acid equivalent/g for M/C extracts and 432.19 to 0.93 mg/g in case of aq. extracts , Free radical scavenging varied from 94.9-14.38% for M/C extracts and 93-1.04% for aqueous extracts at 100 µg/ml, while total antioxidant potential varied from 287-92.42 mg/g vit. C equivalent for M/C extracts and 201.43-15.09 mg/g vit. C equivalent for aq. extracts. The linear correlation showed that there is a significant correlation between total phenolic content and antioxidant activity. But no significant correlation was found between total flavanoids content and antioxidant activity suggesting the major role of phenolics toward antioxidant potential. On the basis of antioxidant potential, 4 plants including *Phylanthus emblica, Syzijium cuminii, Ficus micocarpa,* and *Jasminum spp* were selected for bio-nanoparticles synthesis. The extract of these plants produced appreciable quantities of silver bio-nanoparticles of the varied size in 24 hrs at optimum concentrations. The bioreduction behaviour of plants and characterization of nanoparticles were carried out by UV-Vis spectophotometry, AFM and XRD analysis. These bio-nanoparticles were tested for their antimicrobial activities against *Salmonella typhi*, *Bordetella bronchiseptica*, *Enterobacter aerogens* , *Escherichia coli* and *Staphylococcus aureus*, while antioxidant activity were evaluated by DPPH free radical scavenging assay. Bio-synthesized nanoparticles shown significant antimicrobial and antioxidant potential.

PP-142

Biological Evaluation, Isolation and Purification of Bioactive Compounds from *Rhazya stricta*Decne

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Medicinally important plants contain a variety of chemical compounds that have been used to treat various diseases in folk therapies. Focus of the current study was upon the isolation, purification and biological evaluation of these components from *Rhazya stricta*. In this study, aerial parts of the plant were crushed and macerated in methanol, chloroform mixture (1:1) followed by solvent-solvent extraction. Initially four primary fractions were made by following the standard protocol of alkaloid extraction from the crude extracts of plants. Chloroformic fraction was continued further for purification on the basis of TLC and bioassay results. Normal phase column chromatography of the chloroformic fraction resulted in the preparation of 21 master fractions, named A to U. Master fraction K, was further proceeded with normal, flash, medium and high pressure column chromatography through bioactivity directed isolation. Finally Ursolic acid was isolated and characterized by using 2D-NMR and LC/MS. The bioassays used in this study include inhibition of TNF-α induced NFκB , aromatase inhibition, inhibition of LPS-induced NO production (nitrite assay), induction of quinone reductase-1, Brine shrimp lethality, Potato disc antitumor assay, antibacterial, antifungal and DPPH free radical scavenging.

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