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Symposium Host - Introduction

The symposium is organized and hosted by the Centre of Excellence in Molecular Biology, University of the Punjab, Lahore Pakistan. In order to build national capability in the new bioscience, University of the Punjab established a nucleus Centre for Advanced studies in Molecular Biology. Molecular Biology Laboratory Complex is spread over 60 acres of land, with covered area of 7000 square meters, including a Laboratory Block, a Teaching Block, a Hostel for PhD Research Scholars. The Laboratory Block is divided into four separate research units comprising a total of 20 Research Labs. and four Conference Rooms, one Production Unit and one Support Facilities Unit comprising a Lab-aid Section (for washing, autoclaving and media preparation), an Animal House, an Insectary, six large Plant Growth Rooms, and storage space for research materials. The Teaching Block consists of a well-equipped Library, Seminar Hall, Photography, Computer Rooms, a Conference Hall, Director's Office, Administration and Accounts Section.

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Dr. Mohsin Ahmad Khan
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Dr. Rashid Bhatti

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Program Schedule

DAY 1 (21 November 2017)

Time	Theme	Speaker
0830-0955 Hrs	**Registration of participants**	
	Inauguration Session	
0955-1000 Hrs	**Guests to be Seated**	
1000-1005 Hrs	**Recitation of Holy Quran**	
1005-1020 Hrs	**Welcome Address**	**Prof. Dr. Tayyab Husnain** Director CEMB
1020-1035 Hrs	**Address by Dean Life Sciences**	**Prof. Dr. Muhammad Naeem Khan**
1035-1050 Hrs	**Address by the Vice Chancellor University of the Punjab**	**Prof. Dr. Zafar Mueen Nasar** Vice Chancellor
1050-1105 Hrs	**Address by the Chief Guest**	**Dr. Farrukh Javed** Provincial Minister of Agriculture
1105-1130 Hrs	**Tea Break**	
	Technical Session	
1130-1215 Hrs	Molecular and physiological approaches for potato improvement	**Dr. Ufuk Demirel** Department Of Agriculture and Genetic Engineering, Omer Helisdemir University, NigdeTyrkey
1215-1245 Hrs	Development of drought and heat stress tolerant Arabidopsis plants	**Dr. Khurram Bashir** RIKEN, Yokohama, Japan
1245-1300 Hrs	**Q/A Session**	
1300-1350 Hrs	**Prayer / Lunch Break**	

Technical Session

1345-14:00 Hrs	Citrus biotechnology: prospectus and limitations	**Ammara Noreen, Assistant Research Officer,** Horticultural Research Station, Bahawalpur
1400-1415 Hrs	Antihyperglycemic and antihyperlipidemic potential of Myrica esculenta	**Shahzada Khurram Iftikhar MPhil Scholar** Centre for Human Genetics Hazara University Mansehra
1415-1435 Hrs	Multivariate analysis of morphometric characters of A. florea populations from District Khairpur in a global context	**Naheed Rajper** University of Karachi
1430-1445 Hrs	Insect bioassay to verify the tolerance level of Helicoverpa armigera on Bt cotton genotypes containing Cry1Ac gene	**Shahid Nazir** Agri. Biotechnology Research Institute, Faisalabad
1445-1510 Hrs	An overview of wheat Biotechnology at Biological Sciences Department of FCCU	**Asma Maqbool** Associate Professor FCCU, LHR
1510-1530	Lignin Down-regulation of Eucalyptus by RNAi	**Muhammad Irfan** Forman Christian College (A Chartered University), Lahore
1530-1550	Studies on Antifungal Potential of Ganoderma lucidum against Alternaria alternate	**Muhammad Asif** IAGS, University of Punjab
1550-1600 Hrs	**Q/A session**	
1600.	**Tea Break**	

DAY II (22 November 2017)

	Technical Session	
0900-0945 Hrs	Confining Insecticidal Gene Expression to Insect Wounding Parts in Transgenic Crops	**Dr. Allah Bakhsh** Omer Helisdemir University, Nigde, Turkey
0945-1015 Hrs	Genome-Wide Analysis Characterization and Evolution of SUC Genes of upland cotton	**Muhammad Abdullah** Anhui Agricultural University, China.
1015-1035 Hrs	Pseudomonas sp. AF-54 exhibits multiple traits of plant growth promotion on Helianthus annuus L. crop of Azad Jammu and Kashmir	**Afshan Majeed** University of Azad, Jammu Kashmir.
1035-1100 Hrs	Replacement of chemical fertilizers by beneficial microorganisms isolated from bovine manure to be used as biofertilizer.	**Dalaq Aiysha** Research scholar, MMG, University of the Punjab
1100-1110 Hrs	**Q / A Session**	
1110-1130 Hrs	**Tea Break**	
	Technical Session	
1130-1215 Hrs	Characterization of Vancomycin-Resistant Enterococcus faecium Capsular Polysaccharide Gene Cluster	**Liaqat Ali** Department of Internal Medicine II, University Hospital Freiburg, Freiburg, Germany
1215-1235 Hrs	Nanoscale Assemblies as Platforms for Encapsulation	**Abid Ali Khan** COMSATS, Islamabad
1235-1300 Hrs	3D structure prediction of βC1 protein and its binding affinity with DCLs as suppressor of RNA silencing	**Khadim Hussain,** Department of Bioinformatics and Biotechnology, GC

	in Arabidopsis thaliana using molecular docking approach	University Faisalabad
1300-1400 Hrs	**Prayer / Lunch Break**	
1400-1600 Hrs	**POSTER SESSION**	
	DAY 3 (23 November 2017)	
09:30-09:45 Hrs	Consanguinity and its association with hereditary disorders in the population of Faisalabad, Punjab, Pakistan	**Dr. Saira Hina** Department of Zoology, Government College Women University Faisalabad, Pakistan
09:45-10:00 Hrs	Novel mutation of irf6 gene in Pakistani Vws individual	**Dr. Saira Malik** Assistant Professor MMG, University of the Punjab, Lahore
10:00-10:15 Hrs	Significance of c-reactive protein and albumin in chronic kidney disease patients	**Dr. Mariam Zameer** Assistant Professor MMG, University of the Punjab, Lahore
10:30-10:45 Hrs	Δ16HER2 positive breast tumors show invasive phenotype and over expression of growth receptors	**Khuram Shahzad** Quaid-i-Azam University, Islamabad
10:45-11:00 Hrs	**Q / A session**	
11:00-11:20 Hrs	**Tea Break**	
11:35-11:50 Hrs	PKB/Akt downregulates tumor suppressor b-cell translocation GENE-2 via inhibition of perk1/2 in leukemia cells	**Dr. Muhammad Imran** Assistant Professor University of Peshawar
1:20-11:35 Hrs	Whole genome expression of epidermis infected with common skin dwellers	**Dr. Sidra Younis** National University of Medical Sciences, Rawalpindi

11:50-12:05 Hrs	Impact of SOCS3 Polymorphisms on Insulin Resistance and Therapeutic Response in HCV Infected Patients	**Rabia Aslam** University of Management and Technology, Lahore
12:05-12:20 Hrs	Determination of TGF- β1 polymorphism at -509 C/T promoter region in HCV patients with and without HCC	**Almina Shafique** Demonstrator (University of Management and Technology, Lahore)- School of Health Sciences (SHS)
12:20-12:35 Hrs	Evidence of 35kd and 30kd Protein to Develop Resistance under Mercury, Zinc, Cadmium Stresses in Cr-resistant Ochrobactrum intermedium	**Uqba Mehmood** Superior University, Lahore
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13:00-14:00 Hrs	**Prayer / Lunch Break**	
14:00-15:00 Hrs	**Closing ceremony**	

ABSTRACT INFO

Session: Stem Cells Research

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Osteogenic differentiation of highly mineralizing human bone marrow-derived msc is modulated by alp, igfbp5, and lrp3 through tgfb signaling pathway

Amer Mahmood, Mona Elsafadi and Musaad Alfayez

Understanding regulatory networks underlying human bone marrow stromal cells (hBMSCs)-lineage differentiation and fate determination is a pre-requisite for their use in therapy. The goal of current study was to unravel the novel role for low-density lipoprotein receptor-related protein 3 (LRP3) in regulating osteogenic and adipogenic differentiation of hBMSCs. Using global gene expression profiling, LRP3 exhibited significant up regulation in the highly osteogenic hBMSC clone (CL1) compared to the less osteogenic clone (CL2) and during osteogenic induction of the CL1 clone. Functional and gene expression data demonstrated LRP3 as a molecular switch promoting hBMSC lineage differentiation into osteoblast and inhibiting adipocytic differentiation. Interestingly, microRNA (miRNA) expression profiling identified miR-4739 as the most down regulated miRNA (-36.11 fold) in the CL1 vs CL2 clone. Using TargetScan prediction algorithm, combined with functional and biochemical assays, LRP3 was identified as a novel gene target for miR-4739, with a single potential binding site for miR-4739 in LRP3 3′ UTR. Regulation of LRP3 expression by miR-4739 was subsequently confirmed by qRT-PCR, western blotting and luciferase assay. Forced expression of miR-4739 mimicked the effects of LRP3 knockdown on promoting adipogenic and suppressing osteogenic differentiation of hBMSCs. Hence, we report for the first time a novel biological role for the LRP3/hsa-miR-4739 axis in balancing osteogenic and adipocytic differentiation of hBMSCs. Our data support potential utilization of miRNA-based therapies in regenerative medicine.

ABSTRACT INFO

Session: Stem Cells Research

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Gene expression analysis of Colon Cancer Associated Transcript 1 (CCAT1) a LncRNA in different grades of breast cancer patients from Punjab

Haleema Sadia*, Zeeshan Javed, Hafiz Fawad Khalid and Mukhtar Ullah*

Breast cancer is the most common type of cancer in Pakistan and every one in nine Pakistani women are suffering from breast cancer. LncRNAs are the genetic sequences that are not transcribed to produce proteins. Colon cancer-associated transcript-1 (CCAT1), a long coding RNA has been reported in the progression of many different types of cancer. The present study was conducted to evaluate the role of CCAT1 in tumor progression of breast cancer in population of Punjab. Fresh formalin fixed tissue samples (grade1-grade3) were collected from Chughtai's Laboratory, Gene expression was performed by Real Time PCR. Relative fold change values of the samples were determined by using Livak method, and statistical analysis was performed. Results indicated that CCAT1 over expression was more common in the age group 31-40 years and patients in Lahore tend to express more CCAT1 expression compared to other regions (ANOVA, p =.002). In similar context, CCAT1 over expression was seemingly higher in Bahawalpur compared to Multan (ANOVA, p =.014). However, there were few samples which showed down regulation of CCAT1 expression. History of patients confirmed that these patients were under chemotherapy and medications. From these findings, it was concluded that CCAT1 is over expressed in almost all grades of breast cancer and certain genetic and epigenetic factors are there which are the primal cause of an increase in over expression. Further studies are required to understand how these factors manipulate gene expression of CCAT1 in Breast cancer.

ABSTRACT INFO

Session: Stem Cells Research

Stem Cell Aging and Antioxidants: Expanding the Possibilities of Cell-based Therapies

Hanaa Iqbal* and Asmat Salim

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Regenerative medicine provides treatment options that can result in restoring the functional and physiological features of the impaired tissue or organ for disorders. Cell-based interventions have been implicated in treatment for a broad spectrum of diseases and are being explored to provide reparative interventions for the diseases which either have only palliative treatments or do not have any treatment option. In cell-based regenerative medicine, stem cells are being widely investigated due to their ability of tissue regeneration, capacity of self-renewal and characteristics of plasticity and differentiation. Though, ideal for cell-based treatment, use of stem cells for different diseases is impeded by several factors. Loss of cells upon transplantation in the impaired tissue is one of these factors that need to be addressed. This loss is due to the increased cell death in the harsh ischemic environment of the damaged tissue. This may be due to the process of aging, as deteriorating regeneration potential is associated with advancing age and with this, stem cells also age. This is accompanied by functional changes in adult stem cells. Moreover, stem cells also go through life-long exposure to several extrinsic threats with advancing age. These insults can accelerate the processes of cellular senescence and cell death. Present study explores the molecular mechanisms associated with the aging of MSCs by comparing certain important parameters in young and aged MSCs. The study is also aimed at evaluating the effects of antioxidants on the aging of MSCs. 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 under 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.

ABSTRACT INFO

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Evaluation of Male Infertility using Hormone Profile and Semen Analyses

Aftab Ali Shah*[1] and Hazir Muhammad[2]

The present study evaluated male infertility by seminal and hormonal analyses. A total of 643 (620 having abnormal semen characteristics and 23 healthy controls) male individuals were included. Semen samples collected from the studied individuals were analyzed for the different parameters; physical as well as microscopic examination following standard protocols of the World Health Organization. Among 620 patients, 33 (5.3 %) were Azoospermic (AZO), 54 (8.71%) were Oligoasthenozoospermic (OASP), 166 (26.77%) were Asthenozoospermic (ASP) and 367 (59.19%) were Normozoospermic (NSCNM). Both total sperm count and sperm motility were significantly low ($P<0.05$) in AZO, OASP and a significant decrease ($P<0.05$) in sperm motility was observed in ASP patients compared to normal individuals. Hormonal profile of essential reproductive hormones including Thyroid stimulating hormone (TSH), Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Testosterone (TST) and Prolactin (PRL) for 23 healthy control, 32 AZO, 54 OASP, 35 ASP and 21 NSCNM patients were also evaluated using ELISA. The TSH and TST values in patients with AZO, ASP and OASP were significantly lower ($P<0.05$) while LH values were significantly higher than that of normal individuals. A significantly higher level of FSH was recorded in patients with AZO, ASP and OASP compared to that of healthy control. An elevated level of PRL was found in all studied male infertility sub-groups compared to healthy individuals. In conclusion, the profiling of serum TSH, FSH, TST, LH, and PRL levels are important for the evaluation of male infertility in humans.

ABSTRACT INFO

Session: Nano Biotechnology

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Antimicrobial activities via cefditoren derived silver nanoparticles

Ghulam Mohyyodin Qumar*, Muhammad Safdar, Sana Zahoor, Yasmeen Junejo*, Rozhgar A. Khailany, Tanveer Hussain, M. Tariq Pervez and Masroor Ellahi Babar

Cefditoren derived silver nanoparticles (AgNPs) were synthesized via a simple one-pot method after mixing AgNO3 and cefditoren with the help of NaOH. UV-Visible (UV-Vis) Spectroscopy and Infrared (FTIR) spectroscopy were used for characterization of AgNPs. Cefditoren and synthesized AgNPs solutions were tested against three gram positive bacterial species like Streptococcus pneumonia (S. pneumonia), Streptococcus pyogenes (S. pyogenes), and Staphylococcus aureus (S. aureus). The data revealed that cefditoren showed activity against S. aureus but none against other species while cefditoren derived AgNPs showed activity against all these three bacteria. These activities were dose dependant when compared to cefditoren. These findings could pave the way for finding the changing behavior associated with other antibiotics after capping AgNPs or other metal nanoparticles and may be used against other resistant strains.

ABSTRACT INFO

Session: Nano Biotechnology

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Sageretia thea (Osbeck.) modulated biosynthesis of NiO nanoparticles and their in vitro pharmacognostic, antioxidant and cytotoxic potential

Ali Talha Khalil* [1,2,3] Muhammad Ali [4], Zabta Khan Shinwari[4]

NiO nanoparticles are biosynthesized using Sageretia thea (Osbeck.) aqueous leave extracts and their biological activities are reported. Nanoparticles (18 nm) were characterized through XRD, ATR-FTIR, EDS, SAED, HR-SEM/TEM and Raman spectroscopy. Antibacterial activity was investigated against six pathogenic bacterial strains (gram positive and gram negative) and their corresponding minimum inhibitory concentrations (MICs) were calculated. UV-exposed nanoparticles were investigated to have reduced MICs relative to the NiO nanoparticles have not been exposed to UV. Moderate linear fungal growth inhibition was observed while Mucor racemosus (percentage inhibition 64% ± 2.30) was found to be most susceptible. Cytotoxicity was confirmed using brine shrimp's lethality assay (IC50 42.60 µg/ml). MTT cytotoxicity was performed against Leishmania tropica-KWH23 promastigotes and amastigotes revealed significant percentage inhibition across the applied concentrations. IC50 values were calculated as 24.13 lg/ml and 26.74 µg/ml for the promastigote and amastigote cultures of Leishmania tropica. NiO nanoparticles were found. Moderate, antioxidant potential was concluded through assays like DPPH, TAP and TAC. Furthermore, protein kinase inhibition and alpha amylase inhibition is also reported.

ABSTRACT INFO

Session: Functional Genomics

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Assessment of Antioxidative Status and Circulating Biochemical Markers to unshedow the causative and risk factors associated with Ethambutol Induced Toxic Neuropathy (TON)

Khuram Aziz*[1], Kamran Arzoo[2], Muhammad Naeem Rustam[3], Muhammad Usman Ghani*[4], Arif Malik[5] and Shahzad Ahmad[6]

Ethambutol (ETM) is used in treating tuberculosis and 2-6% of under treatment patients receiving this anti-mycobacterial drug develop toxic optic-neuropathy. The current study was designed to assess the potential role of circulation biochemical markers and antioxidative status in development of EMB induced toxic-optic neuropathy. Thirty five patients undergoing EMB therapy for tuberculosis and twenty age-matched healthy-controls were recruited for study. Patients received a daily dose of 25mg/kg EMB in first two months and 15 mg/kg for the remaining therapy duration. The results predicted a clear cut difference regarding circulating biochemical markers and antioxidative profile among control and patients. The data analysis concerned with hematology (Hb, p-value 0.02; RBC, p-value0.021), renal (creatinine, p-value0.001; urea, p-value0.03), hepatic (Total-bilirubin, p-value0.01), lipid (triglycerides p-value0.03; cholesterol p-value0.01) and anti-oxidative (catalase, p-value0.02; glutathione p-value0.02; superoxide dismutase, p-value0.005, Malondialdehyde, p-value0.02) profiles predicted a significant difference in patient and healthy controls. Vitamin-A,B1, B12, E and Zinc looked to be involved in the induction of induced toxic-optic neuropathy, specially Vita-B1 and E which superpass other antioxidants because of high significance inverse-relationships with Malondialdehyde [Malondialdehyde vs VitaminB1, r= -0.724**; Malondialdehyde vs VitaminE, r = -0.676**]. Our study suggest that during EMP therapy, decreased peripheral level of Vitamin B1 and E may be an inducing factor for EMB induced toxic-optic neuropathy and these vitamins might be therapeutic agents to minimize the toxic effects of ethanbutol drug.

ABSTRACT INFO

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rs1837253, rs1805010 and rs2495636 SNPs as asthma risk factors in Pakistan

Ifrah Khalid, Muhammad Usman Ghani, Iqbal Bano, Ahsan Waheed Rathore, Mariam Shahid, Alishba Maryam, Muhammad Akram and Muhammad Farooq Sabar*

Asthma is a chronic airways disorder which affects the people of all ages and 3rd leading cause of hospitalization in children worldwide. Both genetic and environmental factors are involved in asthma pathogenesis. Numerous asthma genetic association studies have been done in different populations but rarely in Pakistani population. Therefore, the current study was designed to find any association of three selected SNPs (discussed below) with asthma in local population of Lahore region, Pakistan. SNPs rs1837253, rs1805010 and rs2495636 present on Thymic stromal lymphopoietin (TSLP), interleukin-4 receptor α (IL-4Rα) and interleukin-13 receptor α 1 (IL-13Rα1) genes respectively were selected for the study. The selection preference was given to these SNPs due to their association in different ethnic groups but not specifically with Pakistani population. These SNPs were analyzed by SNaPshot or Mini-sequencing technique. SHEsis software was used for statistical analysis. The results revealed that rs1837253 might be significantly associated at allelic model (P=0.01) and trending towards asthma at genotype model (P=0.06). The other SNP i.e., rs1805010 is reported here as not associated with asthma both at allelic and genotype model with P-value more than 0.05. The third SNP rs2495636 presenting a trend towards asthma at allelic model (P=0.06) and a significant association at genotypic model (P=0.002). The variability in results compared with other ethnic groups might be due to different experimental scenario, environmental conditions and sample size. In conclusion rs1837253 and rs2495636 poses risk towards asthma in Lahore Pakistan, which need to be confirmed by large sample size.

ABSTRACT INFO

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CRISPR/Cas9 Based Genome Editing System Opening up a New Era in functional Genomic of Polyploid Crops

Shabbiir Hussain* and Madiha Habib*

World had entered in an era of functional genomics and now the scientist are interested in genomics of polyploid crops with relatively bigger genomes. Scientist are also interested in genomics of polygenic traits especially in polyploid crops such as wheat, cotton etc. During last few decades' different breeding and mutagenic techniques were employed for crop improvements with respect to polygenic traits but the success stories are limited. Various conventional, physical, chemical and biological mutagenic agents were utilized to determine the contribution of major and minor genes controlling complex polygenic traits in different crops. Every strategy have its own pros and cons. Most of them are random in nature and require sequence information as well as needed large screening population and screening time. Presently NGS opened up a new corridor to explore the functional genomics of polygenic traits of polyploid plants. Polyploid crops have tremendous ability to buffer against the induced mutation due to the presence of multiple genome in a single plant. The homoeologous effect of other genome mask the effect of mutated one. Now with the advances in site directed mutagenesis Crispr Cas/9 system will be an influential tool to induce mutation in multiple genomes at a time and will help to break the genetic redundancy/ buffering capacity of polyploid crops against mutation. This tool will also help to explore the multigenic traits as well as the effect of minor genes contributing in complex traits.

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Expression profile of HIF-1α and MDR1 in understanding the dynamics of chemotherapy resistance in breast cancer patients

Zaira Rehman and Hajra Sadia*

Breast cancer is one of the important and leading cause of deaths among women in Pakistan. Genetic and environmental factors contribute to etiology of breast cancer. Although extensive literature is available regarding the genetic screening of breast cancer, their sensitivity and specificity and their ease of use in clinical settings varied globally. This implies requirement of further more identification of screening markers that can help in the management of chemotherapy receiving cohort. Current study is based on the expression analysis of HIF-1α and MDR1 in blood specimens of breast cancer patients in an attempt to identify some blood based marker of tumor progression and chemotherapy resistance. Expression of HIF-1α and MDR1 was studied through real time PCR analysis in blood of seventy two breast cancer patients. Data was normalized through GAPDH. Correlation of clinicopathological characteristics with expression of HIF-1α and MDR1 was studied through Fisher's Exact test in SPSS. There is high level of HIF-1α and MDR1 expression in blood of breast cancer patients. Significant correlation of HIF-1α expression was observed with TNM stage ($p<0.005$), metastasis ($p<0.05$) and chemotherapy status ($p<0.005$). There was no significant correlation of MDR1 expression was observed with age, tumor stage and metastasis. High level of MDR1 expression was observed in patients who are taking chemotherapy as compared to treatment naïve patients ($p<0.05$).
From the current study it can be concluded that the dynamics of HIF-1α and MDR1 expression monitoring may be useful in the management of chemotherapy resistance.

ABSTRACT INFO

Session: Functional Genomics

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Identification and Characterization of ESTs from Zea mays L. through DNA Microarray under Different Abiotic Stresses

Moon Sajid*, Mariam Riaz, Bushra Rashid, Sameera Hassan, Zarnab Ahmad, Muhammad Bilal Sarwar and Tayyab Husnain

Maize (*Zea mays* L.) is one of the most important cereals due to its applications from animal food to industry. Present study was planned to analyze the effect of environmental extremes on maize seedlings at molecular level by using DNA microarray. Microarray is a latest technique to analyze the whole cell transcriptome at one time. Maize seedlings at three leaf stage were subjected to 45°C, 4°C, 10%PEG and 200mM NaCl for a period of three hours and leaf and root samples were collected for RNA isolation. cDNA has been synthesized, labelled with Cye-3 and Cye-5 dyes and hybridized with maize-specific oligo microarray slides. Slides were scanned and relative intensities of spots have been analyzed for differential gene expression (DGE) analysis. Gene ontology analysis has shown that most of the genes expressing under stress belong to proteins kinases family and transcription factors like zinc fingers, basic helix loop helix, auxin responsive factors and TATA box binding protein associated factors. Differential expression of many gene including sugar transporters, ubiquitin ligase, DNA and RNA binding and cytochrome P450 domains that prepare plant to tolerate stress have been also observed. It is concluded that Z. mays L. is naturally equipped with many stress tolerant gene sets and tolerance to abiotic stresses can be enhanced if these genetic units are properly regulated.

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Genome Sequence And Genome Wide Comparative Analysis Of Drug Resistance Genes In Mycobacterium Tuberculosis Resourced From Pakistan

Asma Muhammad Yar, Ghunva Zaman and Muhammad Ibrahim*

Globally tuberculosis (TB) is the second main cause of death, the causative agent of the disease is M. tuberculosis which is multidrug-resistant and kills almost 3 million people worldwide annually. Whole genome sequence (WGS) study could be helpful to understand the drug resistance pattern by identifying the genes involved in virulence and resistance of M. tuberculosis to drugs particularly in Pakistan where until now no genome has been sequenced. In the present study, we did identification of M. tuberculosis isolated from patients in Pakistan by 16s RNA genes and phylogenic analysis following whole genome sequence of M. tuberculosis by using Illumina MiSeq 300PE throughput 2M. This was followed by genome assembly, genome annotation, comparative genomics analysis, and prediction of drug resistance genes and the validation of drug resistance genes by real time PCR analysis. The size of assembled genome is 4,409,295 bp, containing 4305 coding sequences and 48 RNAs. This study resulted in the identification of the drug resistant genes like katG, inhA, ahpC, fabG, rpoA, rpoB, rpoC, against first line isoniazid and rifampicin drugs. The drug resistant genes that were previously characterized on the basis of molecular features or cellular localization was now computationally characterized which resulted in the identification of domain like PRK, fabG and PKX. These genes belong to different protein families like adh_short_C2, peroxidase, AhpC-TSA RNA_pol_Rpb1_1 and RNA_pol_L. The transcript profile of selected genes revealed that several genes didn't respond to drugs induced reveal that might be these genes may not be used as molecular marker for M. tuberculosis strain MNPK or may have different drug resistance pathways. The identification of drug target and virulent genes could provide valuable information to fight this old foe.

ABSTRACT INFO

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Optimization of DNA Isolation protocol of Katydids (Orthoptera: Tettigoniidae) with its morphological aspects

Waheed Ali Panhwar*[1], Riffat Sultana[2] and Muhammad Saeed Wagan[2]

Pakistan is a biogeographically diverse region mainly due to its rich vegetation and favorable climatic condition which made it trouble-free region for breeding of different insects groups including grasshoppers. Tettigonioidea are phytophagous insects some of the species are important pests of agricultural crops while many species are ecologically associated with forest biocenoses, damaging trees and shrubs. In addition to herbaceous plants, these facts extend the range of injurious plants to forest, fruit orchards, berry shrubs and grasses. Because of this work, a total of 4379 adults were collected from different climatic zone of country during the year 2011-2016 and field sites included: agriculture land, forests, fruit orchards, grapevine, berry shrubs, hilly, semi desert and desert areas, trees, shrubs, herbs and grasses. The collected material was sorted out into 47 species of Tettigonioidea pertaining to 07 sub-families i-e: Pseudophyllinae, Phaneropterinae, Conocephalinae, Tettigoniinae, Hexacentrinae, Mecopodinae and Decticinae belonging to 20 tribes, 22 genera, and constricted 29 new records to Pakistan and 05 new species to science. Details for the identification included valid names, their synonyms, measurement of body length, wing span, habitat and morphological description are provided. A simple and cheap genomic DNA isolation protocol for Katydids was developed that will help for further molecular studies in future. It is concluded that there is a vast diversity to explain katydid fauna of this region. Further research should be carried out with larger population size for biodiversity.

ABSTRACT INFO

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Multivariate analysis of morphometric characters of A. florea populations from District Khairpur in a global context

Naheed Rajper*, Naheed Rajper* and Shakeel Farooqi

Current study addresses partitioning the morphometric variation within and between honey bee populations and finds the relationship between them. The morphometric data of honeybee sub-species from different corners of world has been re-evaluated. For this purpose morphometric data of honeybee from other studies as well as from Oberursel, Germany have been taken and compared with the data obtained from District Khairpur. A total of fourteen characters of one hundred worker bees from Khairpur were studied and only common characters from Khairpur and world datasets were used for comparative study. The data was organized and analyzed by Multivariate Statistical Analysis, Mean, Standard deviation, Principle Component Analysis (PCA), Discriminant Function Analysis (DFA) and Cluster analysis (CA). Statistical results showed that significant differences were present in Khairpur honey bee populations and other honey bee data sets from Ilam, Khuzestan, Bushehr, Hormuzgan, Cambodia, N. India, S. India, Srilanka, Iran, Mayanmar, Nepal, Oman, Pakistan, Thailand and Vietnam.

ABSTRACT INFO

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Studies on Expression, Purification and Characterization of Codon Optimized HSA-CIFN Fusion Gene in Pichia pastoris

Muhammad Umair Naseem*, Nadeem Ahmed*, Saad Tahir, Mohsin Ahmad Khan, Ahmed Usman Zafar and Tayyab Husnain

Consensus interferon (CIFN) is a synthetic small messenger molecule which has strong antiviral potential as well as antiproliferative activities. Due to its short blood circulating half-life in human body, it is injected frequently. Unmodified and PEGylated CIFN is injected thrice and once in a week respectively for 6 months to 12 months to cure hepatitis C infection. Human serum albumin (HSA) has long circulating half-life and it is fused with small peptide cytokines to improve their serum stability. In present study, for the production of potent long lasting CIFN, it was fused with HSA via a flexible linker. A codon optimized HSA-CIFN fusion gene was synthesized and cloned into pPICZα B vector under AOX1 promoter. HSA-CIFN/pPICZα B expression cassette was integrated into Pichia pastoris genome. Positive and stable P. pastoris clones were selected through Zeocin™ inhibition screen and colony PCR. HSA-CIFN fusion protein was expressed by inducing the biomass of positive P. pastoris clone using 0.5% methanol for 96 hours. SDS-PAGE was performed to identify the presence and size of fusion protein in the culture broth. Protein was quantified by Bradford assay. One step purification of HSA-CIFN fusion protein was performed by affinity chromatography. Purified fusion protein was analyzed by SEC-HPLC and western blotting was done using anti-interferon antibody. SDS-PAGE confirmed the presence of 87kDa HSA-CIFN fusion protein and its concentration in culture broth was 250 mg/L. More than 98% purity with 68 mg/L yield of HSA-CIFN fusion protein was achieved by one step purification as assessed by SEC-HPLC. Further, western blot analysis revealed the presence of specific purified protein.

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Husnain Qamar

Antimicrobial potential assessment of Pakistani wild Plants

Husnain Qamar, Kausar Basit* and Rashid Bhatti

Screening of medicinal plants is a pre-requisite to analyze their therapeutic potential activity and it can lead the isolation of new bioactive compounds in these plants. Methicillin-resistant Staphylococcus aureus (MRSA) is responsible for skin infections in human. MRSA can infect both non-surgical and surgical wounds. In humans, Enterococcus faecalis cause different types of infections like meningitis, septicemia, endocarditis and urinary tract infections. Root canal treated teeth are more prone to infection by this bacterium. It is more pathogenic in hospitals as it acquires the resistance against different antibiotics. S. pneumoniae is not only the primary reason of pneumonia but is also responsible for other diseases like pericarditis, meningitis, bronchitis, brain abscess and osteomyelitis. S. pyogenes cause both systemic diseases and skin infections. In the beginning, infection occurs on the skin or in the throat then spread. These microbes are developing resistance against antibiotics due to overuse of antimicrobial drugs. Because of this resistance treatment is becoming difficult. Moreover, the available antibiotics are also having serious adverse effects. In this scenario, there is a need to discover the alternative antimicrobial drugs for infections caused by above bacteria that should be efficient and cost-effective. In this study, ten wild plants were collected from different areas of Pakistan and extracts were prepared in methanol. Antibacterial activity of these extracts at various concentrations was assessed against MRSA, Enterococcus faecalis, S. pneumoniae, and S. pyogenes by disc diffusion method. Out of ten plants, six plants exhibit the antimicrobial activity. All the four bacteria are susceptible to these six plants while they are resistant to remaining four plants. These potential antibacterial plants may be used in the development of efficient and cost-effective drugs against above mentioned bacteria after further research in future.

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Nanoscale Assemblies as Platforms for Encapsulation

Abid Ali Khan*[1], Ayesha Talib[1], Ayesha Ejaz[1] and Mohamad Roji Sarmidi[2]

Encapsulation of drugs, bio-active reagents, molecules and nanoparticles in nanoscale platforms is one of the most promising approaches in the development of nanomedicine that provides for efficient drug loading along with reducing systemic toxicity. Moreover, smart targeting of these nano-vehicular systems can greatly enhance the accumulation of the drug at its desired action site(s). Drugs encapsulated in nanovehicular systems exhibit improved bioavailability, biodistribution and activity relative to nonencapsulated counterparts. In this work, we report encapsulation of drug(s) and natural (refined) bioactive reagents for many different purposes. Tobacco Mosaic Virus(TMV) is a rod-shaped plant virus that is 300 nm long and 18 nm in diameter with a 4-nm channel insde it. The plant virus had been encapsulated with Pt(II)-containing drugs followed by the effusion of it from the virus. Our group also encapsulated Virgin Coconut Oil (VCO) in Solid Lipid Nano-Particles (SLP). VCO loaded SLP showed improved skin penetration as well as better moisturization of the skin. Niosomes are the next generation of Nano-structured Lipid Carriers (NLC) that can be synthesized from nonionic surfactants and cholesterol. Encapsulation of VCO along with emulsifying agents and nanoparticles showed great potential to inhibit growth of Multidrug Resistant (MDR) bacteria especially Staphylococcus aureus. The drug encapsulated niosomes gave extended release of the drug, which could result in decreased dose, lesser days of treatment and more patient compliance.

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Impact of SOCS3 Polymorphisms on Insulin Resistance and Therapeutic Response in HCV Infected Patients

Rabia Aslam, Nadeem Afzal and Saba Khaliq*

Suppressor of Cytokine Signaling 3 (SOCS3)gene belongs to SOCS family as one of the negative regulators of cytokine signaling and IFN response that function via the JAK-STAT pathway in antiviral response. SOCS3 expression and genetic polymorphism influences the pathogenesis and outcome of antiviral treatment in hepatitis C virus (HCV) infected patients. This study was designed for analysis of SOCS3 gene expression and polymorphism in Pakistani HCV patients. This descriptive study was conducted on 57 diagnosed HCV genotype 3a infected subjects. The study population was divided into two major groups on the basis of therapeutic response i.e. early virological response (EVR) and relapsers. SOCS3 gene mRNA expression analysis was done by using Real time PCR technique, whereas ARMS PCR technique was used for analysis of SOCS3 gene polymorphisms i.e. 8464 A/C (rs12952093), -4874 A/G (rs4969170) and -1383 A/G, (rs4969168). Gene expression analysis of SOCS3 showed that there was statistically significant increase of 1.92 ± 1.28, and 3.72 ± 1.08 folds in relative gene expression for EVR and relapsers as compared to normal healthy samples ($p < 0.001$). The distribution of rs4969168, rs4969170 and rs12952093 genotype frequencies between EVR vs relapsers group were not statistically significant, only the allelic frequency of rs4969168 was statistically significant ($p = 0.013$) with therapeutic response. The gene expression analysis of SOCS3 showed a clear difference in mRNA expression of SOCS3 as an indicator of therapeutic response rather than polymorphism of SOCS3 gene in our studied population.

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Effect of lauric aicd on health of NDV infected broiler chicks

Nasir Mahmood*, Farzana Rizvi, M. Kashif Saleemi, M. Wasim Usmani, M. Zulqarnain Shakir and Jahanzeb Tahir

Coconut oil can be said as a super food due to its unique characteristics. About 50% lauric acid is present in coconut oil. In body lauric acid is converted into monolaurin, a monoglyceride having antimicrobial property. Lauric acid exhibits antiviral activity against many enveloped viruses such as NDV by inhibiting their penetration and maturation also disturbing there membrane and interfering virus assembly. Lauric acid was given (1, 2 and 3 ml/L of water) to broiler chicks to determine its antiviral property. At the same time control negative and positives group of broiler chicks were kept for comparative study NDV was isolated from filed outbreaks and its velogenic strain was determined by Multiplex RT-PCR. At 18th day of age birds were inoculated by velogenic strain NDV (ELD50 10-5.46). Mortality and morbidity was reduced in broiler chicks receiving higher dose of lauric acid during the disease. There was non-significant effect of lauric acid on hematological parameter of broiler chicks of all groups. Development of lesion of NDV like proventriculus hemorrhages and ulcer in intestine was quite prominent positive group as compared to lauric acid treated NDV infected birds from this study it can be concluded that 3ml/L of water lauric acid help to reduce the losses due to viral infections.

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Lauric acid as immune modulatory agent against Newcastle disease virus

M. Wasim Usmani*, Farzana Rizvi, M. Kashif Saleemi, Nasir Mahmood, M. Zulqarnain Shakir and Sadaf Faiz

Lauric acid and glycerin are byproducts of coconut fat having antimicrobial activity. It has also immune modulatory property. Newcastle Disease Virus was isolated from field outbreaks. The virus was confirmed through RT-PCR was inoculated in broiler chicks to produce infection. One hundred and twenty five birds were randomly divided into 5 groups, group A & B served as negative and positive control. Lauric acid at dose rate of 1, 2 and 3 ml/L of water was given to rest of the three groups. At 18th day of age, 0.1 ml/bird velogenic strain (ELD50 10-5.46) was injected s/c in all birds except control negative. During trial, mortality and morbidity were significantly lower in highest dose rate group. Body weight, feed intake, absolute and relative weight of lymphoid organs was significantly higher in control negative and 2ml/L treated group. Antibody titre against SRBCs was significantly higher in broiler chick supplemented 3ml/L of lauric acid as compared to the rest of groups. Cellular response against tuberculin and phagocytic index was significantly improved at 3ml/L of lauric acid. From this study, it is concluded that lauric acid has beneficial effect on health and improve the immune status which enables the birds to fight against common pathogens.

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Determination of TGF- β1 polymorphism at -509 C/T promoter region in HCV patients with and without HCC

Almina Shafiq*, ShahJahan[2], Nadeem Afzal[3]

HCV is a major health prob;em people facing in Paksitan. HCV-associated liver diseases range from chronic hepatitis to fibrosis, cirrhosis and HCC. Polymorphisms of cytokines genes affect host response to viruses. TGF β1 is a suppressor of tumor initiation by inhibiting cellular proliferation or by promoting cellular differentiation or apoptosis in the early phase of cancer development. This study was aimed to determine the polymorphism in the promoter region of TGF-β1 gene that leads to development of HCC in HCV patients. It was a comparative study, comprising of 80 individuals who were divided into two groups of 40 subjects in each. Group 1 composed of chronic HCV patients while group II had HCV patients with HCC. TGF-β1 polymorphism was determined by RFLP following conventional PCR. Data was analyzed using SPSS 20.0. CT genotype frequency and percentage was detected as 11(39.3%) and 17(60.7%) with [OR (95%CI) =1.422 (0.457- 4.427), P= 0.544) respectively. While TT genotype was found with frequency and percentage of 16(53.5%) and 14(46.7%) with [OR (95%CI =2.511 (0.786-8.029), P=0.120] respectively. These results show that there is no significant association between these polymorphisms in HCV and HCC patients but the patients possessing TT genotype or at least having 1 T allele might be at risk of developing HCC due to high OR compared with CC genotype. There was no statistically significant association in the frequencies of allele (TT, CT, CC) TGF-β1 at -509 C/T promoter region in HCV and HCV patients with HCC. While patients carrying the TT genotype or carrying at least 1 T allele had high OR than carrying the C allele.So, TGF-β1 -509 gene polymorphism might be associated with the risk of HCC in patients with chronic HCV infection in local population.

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Sequence analysis of HPV16 E5 oncogene from Pakistani cervical cancer isolates

Naureen Ilahi* and Attya Bhatti

Worldwide HPV-16 is the considered the most prevalent high risk HPV type. HPV E5 has been reported to take part in early oncogenic events. Molecular variants of E5 gene have previously been reported that could affect its oncogenic potential. Lack of local data in this regards prompted this study. Thus we aimed to examine the E5 gene sequence in HPV16 harboring cervical cancer samples in our population. Extracted DNA from HPV-16 positive formalin fixed paraffin embedded cervical cancer tissue biopsies (n=11) was subjected to HPV 16 E5 gene amplification through PCR. PCR product was purified, sequenced and analyzed using bioinformatics programs. Each of the two cervical cancer samples (Isolate 10 and 11) were found to have two independent point mutations. Three of these mutations were found to be silent mutations while one non-synonymous mutation in isolate 11 was found to replace leucine (L) at position 44 by Isoleucine (I) amino acid. This is the first study reporting HPV16 E5 gene sequence from Pakistan. Most of the point mutations observed were found to be silent showing E5 sequence conservation at the amino acid level among the isolates. Phylogenetic analysis based on E5 gene sequence revealed that HPV16 in Pakistan was closely related with European lineage. In general, European variants are considered to be more prevalent and associated with severe cervical dysplasia or carcinoma. A high prevalence of European lineage in the Isolates indicate a possible epidemiological linkage between Europe and Pakistan with regard to the dissemination of HPV16 infections in Pakistan. Moreover since the isolates in our population resembled variants with more tendency of dysplasia, contained mammalian codons and belonged to European lineage, we can infer that the HPV E5 in our local population is more likely to be efficiently translated and provides support to HPV in causing cervical cancer.

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BST-2/Tetherin potentiates motility of virus infected cells: Potential for virus dissemination

Wasifa Naushad* and Hajra Sadia

The restriction factor BST-2/tetherin plays a critical role in preventing the release of progeny virions from infected cells and in promoting viral clearance through activation of signal transduction pathways. Although BST-2-mediated effect limits cell-free virus infection and spread, what happens to the infected cells ladened with unreleased virions is yet to be determined. We hypothesize that BST-2-tethered viruses may alter the molecular signature of infected cells thereby regulating cell-to-cell viral spread. Using acute retrovirus infection model and various cell lines (cervical epithelia-derived TZM-bl, breast-epithelia-derived 4T1 cells, monocytic U937, and SUP T cells), we show that the titer of extracellular virus significantly increases while intracellular viral load decreases in BST-2-suppressed cells compared to their BST-2-expressing counterparts. Despite equivalent starting cell numbers, BST-2-expressing cells exhibit increased proteolytic and non-proteolytic cell motility. The motile cells contain high levels of viral nucleic acids, BST-2, and metalloproteases. Interestingly, increased cell motility of BST-2-expressing cells correlates with increased infection of target cells in a trans-infection model of virus transmission. We conclude that BST-2 may promote virus spread by enhancing the ability of virus-ladened cells to migrate, carve through extracellular membranes, and infect a population of target cells at distal sites.

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Molecular detection of TEM and CTX-M genes in β-lactamase producing Gram-negative rods isolated from clinical specimens of Pakistani population

Khadija Tahira[1], Faqeeha Javed*[2], Madeeha Javed*[3], Sidra Saleem[1], Muhammad Shafique[2] and Shah Jahan[1]

ESBL (Extended spectrum beta lactamases) producers are tremendously broad spectrum β-lactamase enzyme possesing bacteria, found in the broad range of Enterobacteriaceae. Infection due to ESBL producers ranges from minor UTI (urinary tract infection) to life threatening sepsis. ESBL are readily changing enzymes posing a serious peril in treatment of community and hospitalized patients. We aimed to investigate the ESBL positive clinical specimens by phenotypic screening and confirmatory methods, along with molecular detection of TEM (Temoneira gene) and CTX-M genes (Cefotaxime resistant gene) accountable for the ESBL phenomenon. A total of 70 presumptive clinical isolates collected from a tertiary care hospital of Lahore from the following specimens; urine (n=28), pus (n=24), catheter tips (n=5), blood (n=4), stool (n=4), ear swabs (n=3) and broncho alveolar levage (n=2). Analytical Profile Index (API-20E) was used for bacterial identification. Antibiogram analysis showed all the isolates were resistant to third and fourth generation cephalosporins. Confirmation of ESBL was performed by synergy between third and fourth generation cephalosporins containing discs (ceftazadime, cefotaxime) and discs containing ceftriaxone-clavulanic acid; about 50 isolates were affirmative to be ESBL. For molecular biological identification, we used forward and reverse primers of TEM and CTX-M genes. It revealed that 19 isolates (38%) possess blaTEM and 10 isolates (20%) carry blaCTX-M. There was also co-existence of blaTEM and blaCTX-M in 5 isolates (10%). We observed that ESBL resistant genes, TEM and CTX-M were prevalent in the inspected population. Thus ESBL producing GNR (Gram negative rods) possesses ability to develop novel resistance mechanisms in the face of newly introduced antimicrobial agents. This is one of the foremost studies reporting enterobacterial isolates producing TEM and CTX-M type ESBLS that are circulating in our population.

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Comparative Study Of Typhoid By ELISA And Widal Test With Blood Culture In Paedriatic Patients In Children's Hospital Lahore

Mariam Zameer*[1] and Farhana Shehzad*[2]

Purpose of the study is to compare the sensitivity and specificity of Widal test and Typhoid (by Elisa IgG and IgM) with the blood culture, in the diagnosis of Typhoid fever. This was a Cross sectional study conducted at Department of Immunology & Serology, The Children's Hospital and institute of child health, Lahore from July 2015 to December 2015. Total 123 blood sample of clinically suspected of Typhoid fever who came in outdoor of Children hospital Lahore, were collected to the immunology & serology LAB of Children Hospital Lahore. Commercially available laboratory methods for detecting Typhoid fever were applied; including enzyme linked immunosorbent assay (ELISA), Agglutination and Blood culture, and results of these techniques were compared.Statistical analysis was done using SPSS 20. Out of 123 clinically diagnosed typhoid cases, 8 (6.5%) were blood culture positive for Salmonella typhi, 26 (21.1) were Widal positive and 46 (37.4%) were Typhiod (by Elisa) positive. Sensitivity, specificity and efficacy of Typhoid (By Elisa) were 87.5%, 66.5% and 67.4% respectively which is greater than Widal test. The p value of chi square for (comparison of widal with culture) was 0.039 and for (comparison of Typhidot with culture) was 0.002, which showed that there was a significant difference between the results of two techniques. Blood culture was taken as gold standard.Conclusion: Blood culture and Widal test are used as conventional methods in the diagnosis of the typhoid fever. Typhoid (by Elisa) test is a new, reliable, specific, easy and rapid serological test introduced commercially for the diagnosis typhoid fever. It offers the advantage of rapid & early diagnosis, with a sensitivity & specificity of 87.5% & 66.0% respectively in culture proven cases.

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Post Transcriptional Gene Silencing of Potato Virus X in *Solanum tuberosum*

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Viruses are found everywhere and cause diseases in a wide range of plant species. Potato, a major food crop in the world, is severely affected by viruses that cause a significant loss in its yield. RNA silencing proves to be an effective technique to develop resistance against plant viruses. Double stranded RNA (dsRNA) which is processed into small interfering RNAs (siRNA) by Dicer, is a key stimulator for RNA silencing to target cognate viral mRNA. In the current study, a short hairpin RNA (shRNA) of 107bp directed against coat protein (CP) gene of potato virus X, was directionally cloned in plant binary vector pCAMBIA1301 under Cauliflower mosaic virus 35S (CaMV35S) constitutive promoter. Recombinant RNAi construct was introduced into potato cultivar Desiree through Agrobacterium. Potential transgenic potato plants were screened through amplification with gene specific primers and through dot blot analysis. Overall, transformation efficiency was 25%. To reveal the potential of RNAi construct, bioassay was performed where transgenic potato plants were challenged with PVX particles. It was found that in one transgenic potato line, mRNA expression of CP-PVX gene was down regulated to 75.50%, while in another line of transgenic potato, the CP-PVX mRNA down regulation was about 70.68% as compared to non-transgenic control. Also, it was observed that no symptom of PVX infection does appear on transgenic potato plants while non-transgenic plants showed maximum symptoms of PVX infection. Conclusively, shRNA renders transgenic potato tolerant towards PVX infection.

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Development of Global Consensus Sequence & Identification Of Conserved Domains In Hcv Ns5b Protein

Khadija Zahid, Zahid Iqbal*, Samia Afzal, Iram Amin, Muhammad Shahid and Muhammad Idrees

Hepatitis C virus (HCV) is one the major threat of liver related infections in developing countries and was first isolated in 1989. The prevalence of HCV in Pakistan is almost 8.3%. The NS5B is HCV RNA dependent RNA polymerase but it lacks the ability of proof reading, therefore high level of genetic heterogeneity found among HCV isolates and it results in 7 genotypes having several subtypes. In addition to extremely high genetic variation, still certain regions in the amino acid residues are highly conserved and can be used as targets for the development of peptide vaccine and antiviral drugs. The present study was designed to amplify and sequence HCV NS5B gene from 3a isolates of Pakistani population. Total 100 HCV ELISA positive serum samples were collected, out of which 77 were also positive for qualitative PCR. After screening 3a HCV genotype positive samples having Ct values >20, 29 sera samples were sequenced for NS5B. Out of these 29 samples, 16 were females and 13 were males having mean age 39.43 +/- 11.75 years (15 to 65 years). The amplified sequences were analyzed through various bioinformatics tools. Almost 76 sequences of HCV NS5B gene belonging to all genotypes were downloaded from NCBI and further used to construct consensus sequence separately for each genotype. All the consensus of 7 genotypes sequences aids in the development of global consensus sequence, which was afterwards translated to peptide sequence and was aligned to provide the enormously conserved regions in all genotype at NS5B protein. This work helped in the selection of 9 extremely conserved peptide sequences that can be used for the development of peptide vaccine.

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3D structure prediction of βC1 protein and its binding affinity with DCLs as suppressor of RNA silencing in Arabidopsis thaliana using molecular docking approach

Khadim Hussain*[1], Ikrma Amad[1], Muhammad Rizwan Javed[1], Nazish Masood*[1], Muhammad Tahir Ul Qamar*[2], Ammara Nasim*[1] and Kamran Rashid*[1]

Begomovirus are the most cataclysmic pathogen of crops globally and cause devastating diseases in tropical and subtropical regions of world. Begomovirus carry an unusual type of circular single-stranded DNA satellite, cited as betasatellite. Betasatellite is about 1.3 to 1.4 kb in size and encodes single protein βC1 that plays pivotal role in viral infectivity by suppressing the function of RNA silencing. The present study was designed to predict the 3-D structure of βC1 protein and to analyze its interaction with dicers like proteins (DCLs) of Arabidopsis thaliana. Structure of βC1 protein was predicted by using I-TASSER web server and then analyzed by using various bioinformatics tools. Structures of different domains of dicer like proteins were also predicted by using I-TASSER. Protein-protein interaction of βC1 protein with different domains of dicer like proteins was studied by using different online servers (ClusPro2) and interacting amino acid residues were identified by using LigPlot, PyMOL and Chimera. RNase and Dicer domains of DCL1 protein were best interacted with βC1 protein. The whole study was conducted to find out the residues and pathways by which βC1 protein takes control over the host defense machinery. Methionine (Met), Tyrosine (Tyr), Threonine (Thr) and Asparagine (Asn) were the most reactive amino acid residues in βC1 protein. These amino acids took part in interactions more often as compare to other amino acid residues. N-terminus portion of the βC1 protein was found more responsive to DCLs as compare to center part or C-terminus part of the βC1 protein. The interaction studies βC1 with different domains of DCL proteins lead to understand the mechanism of pathogenesis of begomovirus complex.

ABSTRACT INFO

Session: Molecular Virology

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Transfusion Transmissible Infections Among Healthy Blood Donors at Blood Bank From Children's Hospital and Institute of Child Health Lahore

Mariam Zameer*[1], Fozia Shfee[2] and Farhana Shehzad*[2]

To determine the frequency of HIV, HBV, HCV, syphilis and malaria in blood donors at Children Hospital & Institute of Child Health (ICH), Lahore and compare with other local and international published data. Place and Duration of Study: This was conducted at the blood bank of Children's Hospital and ICH, Lahore from October 2015 to February 2016. All adult male blood donors who had donated blood during above mentioned period, between 18 to 55 years of age were included in this study. Each and every donor was subjected to a predetermined, prepared questionnaire to find out their eligibility for donation. All blood donors' serum samples were screened for HBsAg, Anti-HCV, syphilis, HIV and malaria by immuno chromatography technique according to manufacturer instruction. Statistical analysis showed that out of 10,048 blood donors, 7.94% (n=798) were infected with any one of the above mentioned diseases and 92.05% (n=9,250) had no infection. The overall frequency of HBsAg, HCV, HIV, syphilis and malaria were found to be 1.59%, 3.75%, 0.11%, 2.08% and 0.39% respectively. The co-infections of HCV + Syphilis, HBsAg + HCV, HBsAg + Syphilis, HCV + malarial parasite (M.P) and HBsAg + HIV + syphilis was 0.12%, 0.11%, 0.01% and 0.0099% respectively. There is a decreasing trend of HBsAg, HCV infections but increasing trend of HIV and syphilis infections in blood donors that is an alarming situation .Thus urgent and vigilant screening of HIV, HBV, HCV, syphilis and malaria is required for blood donors to prevent the transmission of these diseases

ABSTRACT INFO

Session: Genetic Diseases

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Significance Of C-Reactive Protein And Albumin In Chronic Kidney Disease Patients

Mariam Zameer*[1] and Farhana Shehzad*[2]

Chronic kidney disease (CKD) and renal failure (RF) are recognized as significant problems for almost of the last 2 centuries. It is more common in adults than in children. To determine the correlation of CRP and Albumin in chronic kidney disease children. The study was conducted at The Children's Hospital and The Institute of Child Health, Lahore from July 2015 to December 2015, In this cross sectional study a total of 78 patients were included out of which 59 (75.6%) were males and 19 (24.4%) were females, with a male to female ratio of 3:1. The average age was 11.8 with a standard deviation of ±3.06. Serum CRP levels were significantly high in these patients with a mean value of about 21.77 with 16.44 ±SD. Serum albumin levels in these patients were found below normal range giving a mean value of 3.06 with 1.07 ±SD. In chronic kidney disease CRP and albumin are very closely related to each other, as the level of CRP increased the albumin goes on decreasing due to inflammation and decreased synthesis by liver. There was a significant correlation between serum albumin and CRP giving a p-value of less than 0.0001.

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Maternal Risk Factors for Development of Orofacial Clefts in the Child: A Case-Control Study Conducted in Pakistan

Ayisha Ayub*, Anthony H. Bui* and Mairaj Ahmed

Numerous maternal risk factors have been associated with the development of orofacial clefts in children. However, these studies are often limited by methodological challenges and low generalisability, especially with respect to under-resourced settings.This study prospectively evaluated risk factors for development of orofacial clefts in a Pakistani population. A case-control study was conducted at the Cleft Hospital and the Bashir Hospital in Gujrat, Pakistan from December 2015 to December 2016. All new cases of cleft lip and/or cleft palate (CLP) at the Cleft Hospital were included. Sociodemographically similar patients without congenital malformations at the Bashir Hospital were selected as controls. Risk factors associated with CLP were identified through bivariate analyses. Multiple logistic regression was then performed to calculate adjusted odds ratios of developing CLP according to various risk factors. The study included 329 patients with CLP and 131 controls. Upon bivariate analysis, the following factors were associated with CLP: maternal tobacco exposure ($p<0.001$), complications during pregnancy ($p<0.001$), maternal hypertension ($p=0.01$), mother not on any medications ($p<0.001$), mother not receiving vaccinations ($p<0.001$), and lower socioeconomic status ($p<0.001$). Upon multivariate analysis, having a smoking parent (OR=2.09,95% CI 1.22-3.58), and complications during pregnancy (OR=2.38, 95% CI 1.45-3.90) were associated with CLP;mother receiving vaccinations (OR=0.32, 95% CI 0.16-0.64) was a protective factors.The present study provides risk estimates for development of orofacial clefts associated with several important environmental factors. These findings will aid in the development of proper perinatal counseling programs.

ABSTRACT INFO

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Association of Vitamin D and Tuberculosis Patients

Saima Saleem and Waqas Khan*

Tuberculosis (TB) is a transmittable bacterial infectious disease that is top ranked among world's deadliest contagious diseases. Numbers of cases of tuberculosis are increasing in all developed, underdeveloped and developing countries that shows the impact of tuberculosis on world's health. Infection with tuberculosis proves fatal in most cases. Risk due to tuberculosis to global health can be easily understood by the fact that one third of world's population is infected with causative agent of TB, mycobacterium tuberculosis (MTB). It infects human and non-human mammals. M. tuberculosis, M. bovis, M. affricanum, M. cannitii and M. microti and some closely related organisms make up the M. tuberculosis complex (MTB complex). in this study Eighty five blood samples of tuberculosis patients were taken. Tuberculosis was diagnosed by the presence of acid fast bacilli on smear from sputum. Control were drawn randomly from general population. Serum 25 hydroxy vitamin D (25 (OH) D3) was less than <12ng/mL was considered as vitamin D deficiency. ORGENTEC ELISA kit (Carl-zeiss-strabe 49-51. 55129 Mainz. Germany) was used for vitamin D quantification. The results were analyzed by using SPSS software.Mean vitamin D level were 13.9± 1.68 ng/mL in control and 9.3 ±1.38 ng/mL in TB patients. Almost all patients had deficiency of vitamin D level as compared to control. Out of 85 patients 82 patients showed vitamin D level less than 12 ng/mL. Vitamin D deficiency was seen in almost 96% of patients. Female patients (9.05±1.55 ng/mL) also have significantly lower vitamin D level as compare to male patients (9.79±1.34 ng/mL). There was significantly deficiency of vitamin D in tuberculosis patients as compare to control. This deficiency was more pronounced in female and individual with low socioeconomic background.

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Studies of TP53 gene variations in breast cancer patients in Punjabi population and the demographic distribution of patients

Haleema Sadia*[1], Mukhtar Ullah[2], Amna Shafi[2] and Zeeshan Javed[1], Hafiz Fawad Khalid[1]

Alterations in TP53 gene, a tumor suppressor gene has been reported in a number of cancers, including breast cancer. The present study was designed to find the link between TP53 gene mutation and breast cancer progression in Punjabi population of Pakistan. Sixty Modified Radical Mastectomy (MRM) tissue biopsies (sporadic) were collected from different hospitals in Lahore. The project was approved by Ethical review board of Jinnah Hospital Lahore. Ten out of 60 samples were randomly selected and sent to the histopathology laboratory of Surgimid Hospital, Lahore to reconfirm the tumor type and grade. The DNA was extracted from all the 60 breast cancer samples by using the Tiangen DNA extraction Kit. The exons 5-9 of TP53 gene were successfully amplified by PCR and sequencing PCR products were amplified by the ABI 3130X Genetic Analyzer. No mutation was found in exons 5-9 of TP53 gene. The results of this study suggested that exon 5-9 of TP53 gene may have not any contribution in the sporadic breast carcinogenesis of Punjabi Population. The demographic features showed that the breast cancer patients in this study had more Invasive ductal carcinoma, more frequent in older age females, grade 3, rural and low income patients were highest in numbers. There is a need to study a large number of samples, including familial cancers and to perform the sequencing of the complete TP53 gene in both familial and sporadic breast cancer patients. It is also necessary to study the role of other genes of the cell cycle.

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Sunshine Vitamin Deficiency Despite Abundant Sun Exposure

Mahwish Akhtar[1], Rakhshinda Akhtar*[1] and Tanveer Ahmed*[2]

To study the prevalence of Vitamin D deficiency in different age groups and different socioeconomic classes in Karachi. The data will be collected from different laboratories and hospitals of Karachi and survey form will be filled by the patients within the period of 1 month. The subjects will be than 200 of different age groups ranging from infants to elderly persons of both gender in all socioeconomic classes in Karachi. Different statistical parameters will be studied by using SPSS ver. 20 for data analysis. 242 individuals from all over Karachi were examined for vitamin D deficiency and insufficiency that was found to 42.5% and 44% respectably. Patients with chronic use of medicines have lower Vitamin D level than non-users so Vitamin D supplementation should be considered in these patients. Vitamin D deficiency is more common in females than males because of reduced physical activity, low milk consumption and reduced sun exposure. Vitamin D deficiency is more in elderly females than in adolescent girls due to reduced sun exposure of elderly one's. High prevalence of hypovitaminosis D was observed in pregnancy, lactation and infancy. Middle class people may be more vulnerable to Vitamin D deficiency and associated health hazards due to lifestyle factors and their working patterns.

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Frequency Of Celiac Disease In Patients With β -Thalassemia Major

Mariam Zameer*[1] and Farhana Shehzad[2]

Thalasseamia is heterogeneous group of blood disorder that come to pass in autosomal recessive pattern caused by the hemoglobin chains defective synthesis. To determinethe frequency of celiac disease in patients with β-Thalasseamia major. The study was conducted at The Children's Hospital and The Institute of Child Health, Lahore from July 2015 to December 2015. In this cross sectional study, the prevalence of celiac disease in children with β-Thalasseamia major was conducted in a period of 6 months. Patients with β-Thalasseamia major were screened by ELISA technique through anti-tissuetransglutaminase (anti-tTG) IgA and IgG antibodies detection for celiac disease. 83 patients with mean age ± SD 7.87±3.82 were included in the study of which 66.3% were male and 33.7% were female. 9.6% (n=8) patients showed positive screening for (anti-tTG) IgA and IgG antibodies. Borderline cases were 6.0% (n=5) and 84.3% (n=70) patient showed the negative results. Variables were quantified by descriptive analysis. Patients with Thalasseamia major have several symptoms that are well founded with celiac disease. As the prevalence of CD is high in patients with and β-Thalasseamia major patients, so it would be justifiable to screen the patients for CD.

ABSTRACT INFO

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Consanguinity and its association with hereditary disorders in the population of Faisalabad, Punjab, Pakistan

Saira Hina*[1], Saba Khalid[1], Sajid Malik[2] and Noreen Aziz Qureshi[1]

Consanguineous marriages are observed among persons descending from the same common ancestor with close biological relations. There is found high rate of consanguinity in Pakistan, but the information on its relationship with genetic disorders is limited. The present study was done to explore the Prevalence of consanguinity and its relationship with genetic disorders. The present study was conducted in District Faisalabad of Punjab, Pakistan. The consanguinity in Faisalabad was carried out in both urban and rural areas. After consent approval data was achieved from female subjects of the area and differentials in consanguinity rates and inbreeding coefficient (F) were calculated. The prevalence of genetic disorders was also studied and their association with consanguinity was analysed. The consanguinity was not linked with the literacy and origin (urban/rural). When compared Faisalabad with other districts of Punjab regional heterogeneity was observed in the consanguinity pattern demanding further studies.

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Novel Mutation Of Irf6 Gene In Pakistani Vws Individual

Saira Malik*, Saira Malik and Tehreem Farooq

Orofacial clefts including cleft lip and cleft palate are common birth defects, which occur either as a syndrome or as non-syndromic form. Among the common syndromes, which involve cleft lip and/or cleft palate includes Van der Woude syndrome (VWS) and Popliteal pterygium syndrome (PPS). These syndromes are inherited in autosomal dominant fashion and occur due to mutations in different genes including IRF6 (Interferon regulatory factor 6) gene. Major features of VWS include cleft lip, cleft palate, lip pits (in 85% cases), cardiac disorders, missing teeth, and bifid uvula etc. While, PPS involve additional symptoms i.e. skin webbing and abnormal skin around nails. The aim of the current study was to identify the mutations of IRF6 gene in Pakistani VWS and PPS individuals. Four patients of VWS and one with PPS were recruited from different areas of Pakistan and DNA sequencing of the IRF6 gene was performed. We found three known mutations including p.R84C, p.R400W, p.D354N and a novel mutation p.Q435X in the coding region of IRF6 gene in four VWS affected individuals. However, no mutation in the coding exons of IRF6 gene was detected in PPS individual. Our study adds valuable knowledge to the spectrum of IRF6 mutations. This would be helpful in further understanding the disorder and in genetic counseling of the affected families.

ABSTRACT INFO

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Maternal Risk Factors for Development of Orofacial Clefts in the Child: A Case-Control Study Conducted in Pakistan

Ayisha Ayub

Numerous maternal risk factors have been associated with the development of orofacial clefts in children. However, these studies are often limited by methodological challenges and low generalisability, especially with respect to under-resourced settings.This study prospectively evaluated risk factors for development of orofacial clefts in a Pakistani population. A case-control study was conducted at the Cleft Hospital and the Bashir Hospital in Gujrat, Pakistan from December 2015 to December 2016. All new cases of cleft lip and/or cleft palate (CLP) at the Cleft Hospital were included. Sociodemographically similar patients without congenital malformations at the Bashir Hospital were selected as controls. Risk factors associated with CLP were identified through bivariate analyses. Multiple logistic regression was then performed to calculate adjusted odds ratios of developing CLP according to various risk factors. The study included 329 patients with CLP and 131 controls. Upon bivariate analysis, the following factors were associated with CLP: maternal tobacco exposure ($p<0.001$), complications during pregnancy ($p<0.001$), maternal hypertension ($p=0.01$), mother not on any medications ($p<0.001$), mother not receiving vaccinations ($p<0.001$), and lower socioeconomic status ($p<0.001$). Upon multivariate analysis, having a smoking parent (OR=2.09,95% CI 1.22-3.58), and complications during pregnancy (OR=2.38, 95% CI 1.45-3.90) were associated with CLP; mother receiving vaccinations (OR=0.32, 95% CI 0.16-0.64)was a protective factor. The present study provides risk estimates for development of orofacial clefts associated with several important environmental factors. These findings will aid in the development of proper perinatal counseling programs.

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Session: Molecular physiology

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Genotoxic effect caused by Particulate matter (PM 2.5) exposed to coal mines and traffic air pollution at Peshawar, Pakistan

Muhammad Ateeq Qureshi*[1],Fouzia Khan Mehsud[2] and Nida Kazmi[1]

Particulate Matter is the suspension mixture of solid and liquid particles in air. The Particles vary in number, size, shape, surface area, chemical composition, solubility and origin. Therefore, the present study was undertaken to investigate the DNA damage in exposed population. DNA damage in lymphocytes was measured by the comet assay. The results showed that blood PM, DNA damage, superoxide dismutase (SOD) and malondialdehyde (MDA) levels were significantly higher ($p < 0.001$), while glutathione (GSH) level was significantly lower ($p < 0.001$) in exposed groups as compared to control group. In Pearson correlation analysis, blood chromium level showed significant correlation with oxidative stress parameters and DNA damage. The mean tail length of two exposure groups was significantly higher as compared to control. These findings showed that during long-term PM exposure, PM is absorbed in the body, which may be distributed in the various tissues and organs of exposed workers. The present study revealed that occupational exposure to PM can lead to oxidative stress and DNA damage in exposed workers. DNA damage and blood PM level may serve as an efficient biomarker in tannery workers exposed workers.

ABSTRACT INFO

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Pkb/akt downregulates tumor suppressor b-cell translocation gene-2 via inhibition of perk1/2 in leukemia cells

Muhammad Imran*, Mohabat Khan, Hina Zubair and Zahid Khan

Protein Kinase B (PKB), also known as Akt, is a serine/threonine protein kinase. Constitutive activation of PKB/Akt has been demonstrated to be involved in tumorigenesis and is correlated with unfavorable outcome in certain types of cancers. How PKB induces cells transformation and cancer development is largely unknown. Btg2 is a tumor suppressor gene which is implicated in a variety of biological functions. In the current study we report PKB/Akt-mediated suppression of a Btg2 in human leukemia HL-60 and THP-1 cells. RNA was extracted from both HL-60 and THP-1 cells using RNA extraction kit and genes of interest were amplified using both conventional and real time PCR. Proteins expression was determined using Western blotting. The data revealedthat serum deprivation of both HL-60 and THP-1 cells led to a significant ($P>0.01$) increase in Btg2 expression. Employing PKB/Akt inhibitor, LY294002, further upregulated Btg2 expression while inhibiting pErk1/2 using U0126 downregulated Btg2 expression both in the presence and absence of serum, as assessed by PCR. Moreover, immunoblot analysis revealed that PKB inhibition increased activation of pErk1/2 thus depicting PKB/Akt-mediated downregulation of Btg2 via pErk1/2 (MAPK pathway). Furthermore, pErk1/2 was observed to activate NF-κB that acts as a transcription factor for Btg2. Our data shows that PKB/Akt negatively regulates Btg2 expression via inhibition of MAPK NF-κB cascade that may in turn increase cells proliferation and survival hence leading to tumorigenesis. This research uncovers a novel mechanism for PKB-mediated oncogenesis in human leukemia cells.

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Δ16HER2 positive breast tumors show invasive phenotype and over expression of growth receptors

Khuram Shahzad*, Hamid Khan, Aneesa Sultan and Mariam Anees*

Breast cancer is a heterogenous disease with altered molecular signaling often involving growth receptors. 25% breast cancers overexpress human epidermal growth factor receptor 2 (HER2) which is blameworthy for invasiveness and drug resistance in breast cancers owing to its impactful splice variants. This study was undertaken for deciphering the oncogenic and transforming aptness of one of the splice variant of HER2 called Δ16HER2. mRNA level Δ16HER2 expression was probed using RT-PCR with Δ16HER2 specific primers. Immunohistochemistry reports and clinical data was collected from medical records of breast cancer patients. 23 of 35 (65.7%) breast cancer expressed Δ16HER2 of which 91% had invasive phenotype. Δ16HER2 expression correlated meaningfully with breast cancer risk factors including age, number of children and marital status as well as with clinicopathological features such as HER2 receptor status, higher tumor grade, invasive histological subtypes, and age of disease onset and breast cancer symptoms. All these results indicate that HER2 splice variant Δ16HER2 has a potentially important role in the pathogenesis of breast cancer.

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Evidence Of 35kd And 30kd Protein To Develop Resistance Under Mercury, Zinc, Cadmium Stresses In Cr-Resistant Ochrobactrum Intermedium

Uqba Mehmood*

Chromium resistant strain Ochrobactrum intermedium, isolated by Faisal and Hasnain (1998) was used to check their resistance against mercury, zinc and cadmium and their uptake ability of these metals was evaluated. Mercury, zinc and cadmium was estimated in pellet (take up/accumulation), washing of pellet (loosely bound/adsorbed) and supernatant (left over/unavailable) of the strain. Mercury, zinc and cadmium uptake ability of this strain was checked. The strain has great ability to volatilized the mercury. Ochrobactrum intermedium cannot uptake zinc, almost all the zinc was found in supernatant. Ochrobactrum intermedium can uptake cadmium but in low amount. Protein analysis of this strain under metallic stress revealed that they synthesized new polypeptides with different metals. Ochrobactrum intermedium formed protein bands of 35KD and 30KD with Zn, Hg and Cd.

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Bacterial Strains from Tanneries Effluents with Multiple Potentials: Chromium and Azo Dyes Reduction Along with Nitroreductase Activity

Amina Khatoon, Saira Bashir, Tahir Iqbal*, Muhammad Kaleem Sarwar and Asma Nousheen.

Four bacterial strains with Nitroreductase, Chromium and Azo dyes reduction activities were isolated from tannery effluents of tanneries located in Sialkot, Punjab, Pakistan. On the basis of 16S rRNA these strains showed relationship; PSA1 and PSA2 to Bacillus licheniformis, PSA3 to Bacillus anthracis and PSA4 to Bacillaceae bacterium. The growth of bacterial strains, with low hexavalent chromium (Cr6+) reduction, was significantly retorted in the presence of high concentration Cr6+ (100µg/ml). But, high Chromium reduction rate was observed in the presence of 50µg Cr6+/ml, as compared to 100µg Cr6+/ml, after 24 hours incubation. The Azo dye named Methyl red was reduced more efficiently in the presence of glucose and yeast extract as compare to without glucose and yeast extract by the isolated bacterial strains. So, these bacterial isolates with multiple activities may be used as potent biotechnological tools for Leather and Textile industries waste bioremediation.

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Evaluation of Metal-Induced Toxicity and Antioxidant Response in Triticum aestivum L.

Faryal Naeem and Behzad Murtaza*

Large scale urbanization and industrialization during the 20th century has led to high emissions of heavy metals into the ecosystems. Among heavy metals, cadmium (Cd) and lead (Pb) are of concern for food chain owing to their inherent high toxicity even at low concentration to both plants and animals. A pot experiment was conducted to determine soil metal contamination levels sufficient to cause Cd and Pb accumulation in wheat grains, straw and roots and also to study antioxidant activity of wheat to check survival against Cd and Pb toxicity. Soil was spiked with different concentrations of Cd (0.1, 0.2, 0.3 and 0.4 mM) and Pb (2.5, 5.0, 7.5 and 10.0 mM) using CdCl2 and Pb(NO3)2, respectively. To determine combined effect of Cd and Pb, two treatments were spiked with both Cd and Pb concentrations after preliminary pot experiment. The antioxidants (SOD, CAT, APX, POX) response indicated significant ($p<0.05$) increase in enzymes activities during heavy metals stress, hence preventing damage to plants. The highest accumulation of Cd (4.242 mg kg-1) and Pb (763.330 mg kg-1) was found in wheat roots. Maximum grain concentration of Cd (0.923 mg kg-1) was observed at 0.4 mM Cd and maximum Pb (16.340 mg kg-1) was found with 10 mM Pb whereas, concentrations in combined treatment at their minimum and maximum toxic levels was also significant ($p\leq0.05$) with respect to control. Chlorophyll pigments and MSI significantly ($p<0.05$) decreased with increasing metals concentration while MDA increased with increasing metals concentration. The dry weight of roots,shoots and grains, significantly reduced at both Cd and Pb individual levels as well as combined compared to control. This study also highlights the combined effect of Cd and Pb because maximum toxicity symptoms were observed at the highest levels i.e. 0.4 mM Cd and 10 mM Pb and in their combination

ABSTRACT INFO

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Genetic and biochemical profiling of PALB2 within PALB2-BRCA2 interface in Pakistani breast cancer patients

Sumroon Ephraim[1], Hafiz Naveed Shahzad*[2] and Rana Salman Anjum*[1]

Breast cancer is one of the leading reasons of deaths among women in the world. One of the major causes of breast cancer is through ionizing radiations that create DNA double strand breaks. Nature has blessed cells with multiple repair pathways of which homology dependent DNA repair is of central importance. This utilizes recombinase for the homology search and repair. In this repair system, the association of BRCA2 (breast cancer susceptibility type 2) and PALB2 (partner and localizer of BRCA2) is crucial whose disruption can predispose to breast/ovarian syndrome. PALB2 is the successor of BRCA2 in cancer prevention and has been identified to show subtle point mutations in different population based studies. We carried out our studies to investigate the Pakistani population for the identification of possible point mutations in PALB2 within the domain that interacts BRCA2. High quality DNA was isolated from both healthy and tumorous samples which was then employed in PCR based amplification using gene specific primers. Gel extraction method was utilized for the isolation of the PCR amplified gene products from the agarose gels which were then sent for DNA sequencing whose results would be crucial in better understanding of the mutational status of PALB2 in Pakistan. Microscopic slides were also prepared for these samples for histopathological studies that confirmed the cell progression in tumourous cells only. Our studies on PALB2 would help Pakistani population for predisposition of breast cancer.

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Insertion/Deletion of HLA-G Polymorphism in Diabetic Patients having TypeII

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In Diabetic Patient's Cell-mediated immunity was evaluated by the percentage of HLA-G on lymphocytes CD3, CD14 and CD19 by flow cytometry in healthy donors and diabetic patients having type-1. The main aspects of the work were designed to investigate immunological cross-reactivity between viral antigens and pancreas-specific self-antigens.This study evaluates the 14bp Insertion/Deletion (Ins/Del) HLA-G polymorphism in CVD patients. Genotyping of the 14bp Ins/Del HLA-G polymorphism was performed by polymerase chain reaction (PCR). There is also a large variation of cholesterol level for the both groups CVD (235.3 ±9.348) and HC (161.8 ± 3.387). It is also indicated that there is significant difference ($p < 0.0001$) of diabetic patients in glycation level with all other clinical parameters: triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), glucose, protein, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and advanced glycation end-product (AGE) products with the healthy donors. There is significant difference ($p < 0.0001$) in the distribution of HLA-G 14 Ins/Del polymorphism between diabetic patients having CVD and healthy subjects. The frequent regularity of Ins/Del genotype was significantly higher in diabetic patients with CVD compared to the healthy subjects. In conclusion, the study on the significant linkage between the HLA-G Insertion/Insertion genotype and CVD introduces other risk to genetic factors contribution to this common inflammatory disease.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders

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Prevalence of Commonly Reported SNPs in Pakistani Obese Kindreds

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Obesity the accumulation of access fats or access adipose tissues is a major health problem affecting billions of people both in developed and developing countries. Recent report of the WHO reveal, that more than 1.9 billion adults are overweight, and more than 600 million are obese. Spending pattern of financial state and food especially processed food in Pakistan has an important role in the increase prevalence of obesity, type 2 diabetes and cardiovascular disease. Obesity is reported to be caused either due to environmental factors such as food habit, physical inactivity, sedentary life style, and socioeconomic status or due to genetic factors including FTO, MC4R, LEPR, and POMC. Their variants have also been found associated with obesity. Variants of the FTO rs9939609, rs1558902, and rs8050136, Variants of the MC4R rs17782313, rs6567160, and rs12970134, and variant of the POMC rs713586 were analyzed in the present study using ARMS PCR approach. Their prevalence has been investigated to understand the mechanism and causes of obesity in obese individuals from Pakistani population. It was found that none of the already reported variant in our sampled kindred is associated with obesity. It is calculated that environmental and lifestyle factors play more important role in obesity in Pakistani population than genetics.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders

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Association between genetic variant (rs11650680) of TOP2A and early onset asthma in population of Lahore, Pakistan

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rs11650680 is located in the promoter region of TOP2A, a gene residing in the 17q21.2 region. Various genome wide association studies identified the association between rs11650680 and asthma in different ethnic groups. This research work was aimed to study the association of rs11650680 with early onset asthma in a local population of Lahore, Pakistan. Total 143 unrelated subjects (68 patients diagnosed with early onset asthma and 75 healthy controls) were enrolled for this study from Lahore, Pakistan. After DNA extraction by PCI method and amplification of DNA region containing rs11650680 SNP, genotyping was performed in both cases and healthy controls by using minisequencing method. SHEsis and Haploview online software were used to calculate the allele and genotype frequencies. Association analyses were done by using allelic, dominant and recessive genotypic model on PLINK and results were adjusted for urbanization as cofactor using PLINK. This study found heterozygous "CT" genotype of SNP rs11650680 is more prevalent in patients as compared to healthy subjects. "T" allele of this genetic variant is showing a trend towards association with disease in our population. "T" allele of this SNP is significantly associated with early onset asthma independently under recessive genotypic model (p= 0.01947, X2=5.459) and by taking urabinzation as a cofactor (p=0.0183, X2=5.459). The association of genetic marker of TOP2A with early onset asthma is reported in this study. The result demonstrates that epigenetic mechanisms are involved in the development of early onset asthma in patients living in an urban environment.

ABSTRACT INFO

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Whole genome expression of epidermis infected with common skin dwellers

Sidra Younis[1] and Miroslav Blumenberg*[2]

Skin is first line of defense against physical, chemical as well as biological environment. A number of the antibiotics have been produced; however, continuous emergence of resistant strains is a big challenge for human health. Skin is an ideal organ to study molecular responses to biological infections by virtue of diverse skin cells specialized in immune responses. Comparative analysis of skin responses to pathogenic, non-pathogenic and commensal bacteria would help in identification of disease specific pathways for drug targets. In this study, we investigated human breast reduction skin responses to Propionbacterium acnes, Staphylococcus aureus, Staphylococcus epidermidis and TLR1/2 agonist using Affymetrix microarray chips. The Pam3CSK4 solution and bacterial cultures were prepared and inoculated in steel rings that were placed on the acetone treated epidermis in a petri dish. After 24 hrs incubation, 8mm punch biopsies were taken from the center of the ring and RNA was extracted. The genome wide expression was then analyzed using Affymetrix HG-133A gene chip microarray. The bacteria checked cause skin to boost the production of extracellular matrix components and attenuate the expression of differentiation markers. The above responses are mediated via the TLR2 pathway. Skin also responds to S. aureus and P. acnes by inducing the genes of the cell cycle machinery; this response is not TLR2-dependent. S. aureus induces, whereas P. acnes suppresses the genes associated with apoptosis; this is also not TLR2-dependent. The pathogenic behavior of non-resident bacteria is principally defined by their ability to induce host immunity while evading the host immune bactericidal mechanisms through either upregulation of normal cellular processes in infected cells or directly interfering apoptotic process. The commensals have also evolved strategies to survive skin immunity, however they lack ability to initiate immune processes until and unless other factor set a pre-infection environment for them.

ABSTRACT INFO

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Case-control Association Study of HLA-B variant (rs866916063) with asthma in local population of Lahore, Pakistan

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Asthma is an intricate multifactorial disorder comprising both genetic as well as environmental factors. One of the widely studied genetic components in asthma pathogenesis is Major histocompatibility complex (MHC) on chromosome 6p21. SNP rs866916063 resides in the upstream region of HLA-B (Human Leukocyte Antigen-B) an immune regulatory gene in MHC cluster. This study was intended to interpret the association of asthma with rs866916063 in our local population. Total of 115 samples, including 55 physician diagnosed asthma patients and 60 unrelated healthy controls were recruited in this study. DNA from the whole blood was extracted using PCI standard protocol. Amplification and Sanger sequencing of the HLA-B upstream fragment was performed to record allelic variants of targeted SNP. Association analysis of allelic and genotypic models were done with SHEsis plus and results were adjusted with false discovery rate (FDR) corrections. Homozygous alleles "CC" of rs866916063 are highly prevalent among asthmatics (frequency of CC=100%) while the introduction of G allele in genotype results into a protective influence on controls (frequency of CG=11%). According to the findings, rs866916063 is significantly associated with asthma (p=0.008) and association remained significant even after applying FDR correction (p=0.044). This study found that genetic variant present in the promoter region of HLA-B is associated with asthma in Pakistani population. This SNP has not been studied before in any population with reference to asthma. Replication of these findings on a larger sample size can reconfirm these results.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders

Exome sequencing revealed twenty novel pathogenic genetic variations responsible for intellectual disability in Pakistani population

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Intellectual disability (ID) represents group of genetically and clinically heterogeneous disorders. Prevalence of ID is 1-3% in general population. About 50% of ID cases have various genetic etiologies. New research era is focused to identify causative genes for ID and determine underlying mechanism of pathogenicity. Recessive disorders including ID are prevalent in Pakistan. This research was aimed to identify genetic causes of ID in Pakistani population. Next Generation Sequencing (Exome sequencing), new Bioinformatics tools and homozygosity mapping helped to identify pathogenic variations in recurrent and novel genes in this research. Data generated from exome sequencing processed for alignment, variant calling and annotation. A strategy is developed to obtain pathogenic variants from exome sequencing data. These variants are predicted pathogenic by in silico tools. Sanger sequencing confirmed segregation of pathogenic variants in affected members of family. Among recurrent genes include POMT2, SRD5A3, SPG11, AP4M1, MED23, SYNE1, ASPM, ZNF41, PGAP1, DOCK8, ARL13B, ZFYVE26, AP4S1, and MKKS. In addition, novel candidate genes for ID are CAPN12, MDGA2, GPAA1, MEGF9, WFDC1, UBE2J2, CCDC82, TMEM222 and PUS7. Novel ID genes provide new understandings of pathways and mechanisms that could lead to ID.

ABSTRACT INFO

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p.Y556C MUTATION IN SLC26A4 GENE IS A RECURRENT MUTATION IN PUNJABI POPULATION

Sana Zahra*

To understand the interaction between Sbp and Aap, we recombinantly expressed Sbp and a construct (G5EG5EG5) of domain B of Aap after cloning them. The production and purification of miligram quantities of both proteins were done by Ni^{+2} affinity chromatography and gel filteration. After stabilizing proteins in specific buffer, monodisperse properties and the prediction of secondary structures along with the folding states of proteins were analyzed by dynamic light scattering (DLS) and circular dichroism spectroscopy respectively. DLS of the mixture of Sbp and Aap construct demonstrated that they associate with each other in the presence of 5mM ZnCl2. Size exclusion chromatography and native mass spectrometry also confirmed the interaction between them according to their molecular weights. Furthermore, hydrodynamic radius by DLS and radius of gyration of Sbp by Small angle X-ray Scattering (SAXS) confirmed that Sbp oligomerizes with increasing concentrations and thus helps in forming biofilm surface. The ab initio model of Sbp has also been generated by SAXS. We conclude that recombinant, correctly folded Sbp and Aap construct associate with each other. These findings pave the way for the structural characterization of Sbp alone and in complex with Aap which can ultimately help in designing a drug against such a hospital acquired infection.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders

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Downregulation of miR-10a-5p in synoviocytes contributes to TBX5 controlled joint inflammation by influencing apoptosis and proliferation

Nazim Hussain*, Wenhua Zhu

MiRNAs are considered to play critical roles in the pathogenesis of human inflammatory arthritis including rheumatoid arthritis (RA). The purpose of this study was to determine the relationship between miR-10a-5p and TBX5 in synoviocytes and evaluate their contribution in joint inflammation. The expression of miR-10a-5p and TBX5 in synovium of RA and in human synovialoma cell line SW982 stimulated by cytokines were determined by RT-qPCR and Western blotting. The direct interaction between miR-10a-5p and TBX5 3′UTR was determined by dual luciferase reporter assay in 293-T cells. Mimic and inhibitor of miR-10a-5p were transfected to SW982 cells. TBX5 was over expressed by plasmid transfection or knocking down by RNAi. Proinflammatory cytokines, TLR3 and MMP13 expression were determined by RT-qPCR and Western blotting. The effect of miR-10a-5p and TBX5 on apoptosis and proliferation of synoviocytes was also determined. Downregulated expression of miR-10a-5p and upregulation of TBX5 in human RA patients was found compared to OA patients. IL-1β could reduce miR-10a-5p but induce TBX5 expression in SW982 cellsin vitro. The direct target relationship between miR-10a-5p and mRNA of TBX5 was confirmed. Alterations of miR-10-5p after transfection with its mimic and inhibitor caused the related depression and re-expression of TBX5 and inflammatory factors in SW982 cells. Overexpression of TBX5 after pCMV3-TBX5 plasmid transfection significantly promoted the production of TLR3, MMP13 and various inflammatory cytokines, while this effect was rescued after knocking down of TBX5 with its specific siRNA. Down-regulation of miR-10a-5p might involve disturbing the balance between proliferation and apoptosis of synoviocytes by targeting TBX5. We conclude that miR-10a-5p in a relation with TBX5 regulates joint inflammation in arthritis, which would serve as a diagnostic and therapeutic target for RA treatment.

ABSTRACT INFO

Session: Forensic Sciences

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Individualization of shared paternal lineages by using Twelve Rapidly Mutating Y-STRs

Sumbal Sumbal, Muhammad Shafique*, Faqeeha Javed, Ahamd Ali Shahid and Tayyab Husnain

In this research, we used a more profound individualization approach even to discriminate the individuals of shared paternal lineage. We designed a PCR multiplex with our newly synthesized primer sequences for 12 rapidly mutating Y-STRs (DYS570, DYF399S1, DYS547, DYS612, DYF387S1, DYS449, DYS576, DYS5626, DYF403S1a/b, DYS627, DYS526 and DYF404S1). A datasets of 35 pairs with intrinsic paternal relatives like father-son, brother-brother and uncle-nephew were analyzed and run on genetic analyzer by optimizing RM Y-Plex PCR conditions. Polymorphism information content (PIC) for RM Y-STRs was found in the range of 0.720 for DYS576 to 0.870 for DYS627. Maximum allele frequency was observed as 0.410 at locus DYS626 with the allele number 32. All haplotypes of given dataset were found unique in all related pairs except 4 haplotypes which were repeated twice. The overall haplotype diversity was found 0.9794, which represents 98% uniqueness between all selected pairs. Mutation rates of all closely related pairs were observed in the range of 1.90×10-2 to 7.18×10-2 high enough than other conventional Y-STRs which were reported previously between 1×10−4 and 1×10−3. Consequently, rapidly mutating Y-STRs (RM Y-STRs) are highly variable and useful to discriminate a male individual even within the same paternal lineage.

ABSTRACT INFO

Session: Bioinformatics

In silico Drug Repurposing via Chemical-Protein Interaction Analysis: Targeting Pyocyanin based virulence of Antibiotic resistant *Pseudomonas aeruginosa*

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World Health Organization has classified Pseudomonas aeruginosa as one of top priority threat in terms of prevailing pandemic scenario of antibiotic resistant superbugs. This pathogen is widespread in healthcare settings and is listed as one of the top three nosocomial infectious agents. Susceptible population is vulnerable due to lack of vaccine availability to combat this opportunistic pathogen. Pyocyanin (PCN) is considered as prime virulence factor of P. aeruginosa among many others. A wide range of bioactivities have been attributed to this compound primarily based on is redox active nature. We attempted to exploit structural information of PCN in order to screen available drug pool for disrupting or reducing PCN production. PCN molecules structure was studied for potential drug hit via studying chemical-protein interactome (CPI). The CPI data was used to identify query-drug interactions. Screening was performed and high probability hits were selected. The predicted targets were tested for inhibition of PCN production. Peperaquine showed remarkable inhibition of PCN biosynthesis. This predicted reported target may provide a basis for development of a reliable anti-virulence drug against acute and urinary tract infections by P. aeruginosa. The approach adopted here could be extended to other bacterial pathogens for potential immunogenic target predictions and ultimately successful drug development.

ABSTRACT INFO

Session: Bioinformatics

Daidzein and Isoflavone as an anti-obesity therapeutic compound: an in silico approach for structure-based drug designing

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For the inhibition of an enzyme Cholesterol esterase, new drugs are under development which require screening for preclinical and clinical evaluation. The present study deals with the cholesterol esterase inhibitory activity of flavonoids such as isoflavone and daidzein using in silico docking studies. In silico docking studies were carried out using Autodock Version 4.2.6. It was investigated that isoflavone and daidzein blocked the active site apparatus (catalytic triad and oxyanion hole) of human cholesterol esterase (hCEase). In docking study analysis three important parameters including binding energy, inhibition constant and intermolecular energy were determined. The results showed binding energy ranging in between -8.11kcal/mol to -7.45kcal/mol for daidzein and -8.04kcal/mol to -8.01kcal/mol for isoflavone. The intermolecular energy ranging in between -9.0kcal/mol to -8.35kcal/mol and -8.34kcal/mol to 8.31kcal/mol and inhibition constant value of 1.14 µM to 3.45 µM and 1.28 µM to 1.35 µM for daidzein and isoflavone respectively, which coincide with the binding energy. hCEase Inhibitory activity of daidzein and isoflavone could be the result of benzopyran ring in their basic nucleus. These results clearly indicate that flavonoids especially, daidzein and isoflavone possess potential hCEase inhibitory binding sites and further investigations on the above compounds are necessary to develop potential chemical entities for the prevention and treatment of obesity and related disorders.

ABSTRACT INFO

Session:
Bioinformatics

Comparative modelling and docking analysis of mouse serine protease 57(prss57) with crystal structure of human nsp4

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Neutrophil Serine proteases (NSPs) contain active serine proteases; neutrophil elastase (NE), cathepsin G (CG), and proteinase 3 (PR3) and recently discovered Neutrophil Serine Proteinase 4 (NSP4/PRSS57) with 39% identity to NE and PR3. NSP4 is an elastase-related protease in neutrophils with arginine like specificity. In order to prevent the self-induced damage, regulation of proteolysis induced by these serine proteases is carried out by using serine protease inhibitors, serpins. This study shows that mouse PRSS57 and human NSP4 sequences have similar primary structures and the active site residues are conserved. The difference between the models is in the position of aspartic acid (a specificity conferring amino acid) at 226 in humans and 213 in mouse which leads to slight changes in their function. The Ramachandran plot of mouse PRSS57 and Human NSP4 indicates that the main-chain dihedral angles are found in the most favored regions with a Z-score of -7.22 and -6.54 respectively. The quality factor of mouse PRSS57 model is 79.386 and of Human NSP4 crystal structure is 94.366 which indicates the acceptable 3D profile of the modelled protein. There was no significant difference between the two structures, suggesting our model is almost same to the template structure except for the small loops in three regions, α-helices in 2 regions and β-sheet in one region. Proteinase Inhibitor-9 (PI9) and D-Val-Leu-Lys-CMK were successfully docked at the specific site thus blocking the active site formed by the catalytic triad. We conclude that Human NSP4 is a tryptase, therefore, mouse orthologue is also predicted to be a tryptase and possibly involved in same function as performed by human NSP4. Furthermore, being orthologous it seems that the mouse PRSS57 might have a different role as compared to human PRSS57 in order to facilitate specie specific vertebrate's immune function.

ABSTRACT INFO

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Protein Protein Interaction of cag A reveals new drug Targets in Helicobacter pylori

Syeda Quarat Ul Ain, Syeda Marriam Bakhtiar* and Noor Ul Ain

Traditional methods for drug targeting usually skip potential proteins which are not directly involved in pathogenesis but have a strong interaction with key proteins. Literature reveals cagA as key protein in gastric cancer. The cag pathogenicity island in strain 26695 of Helicobacter pylori contains cagA and therefore is known as cagA +strain. This study is designed to find the potential drug targets interacting with cagA protein and their potential role in pathogenesis of gastric cancer. To identify neighbor proteins, manual search on STRING database using the keyword "cagA" was performed, which displayed the extended network comprising 10 proteins which are the functional partners of cagA. Out of which proteins with strong interaction with cagA were selected i.e HP_0527, cagE, cagS, cagT, HP_0525, HP_0524, GP_0522 and flaA. These partner proteins were further investigated to measure the betweenness centrality value and clustering coefficient with the help of pajek tool, on the basis of interacting network it gives the BC and CC values of proteins. It was found that cagA(cai) has the highest BC and CC values which is 0.255952 and 0.714286 respectively. The cagE, cagS, cagT, HP_0524 and HP_0525 are the very immediate partners of cagA in developing risk of gastric cancer because their BC values as well as the degree nodes in the interacting network does not show much difference. cagE, cagS, cagT could be targeted to develop new drugs against H.Pylori.

ABSTRACT INFO

Session: Bioinformatics

Poultry Management System a Bioinformatics Based Solution for Poultry farmers

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The Poultry farmers are becoming aware of use and benefits of information record keeping in their business over the years. However, it is still a neglected farm activity. The ultimate purpose of keeping complete record of farm activities is to improve the overall performance of the farm and reducing the chances of poor performance. Farm management experts frequently cite the importance of good record keeping in improving farm efficiency and profitability. Utilizing more advanced control procedures as well as monitoring the outcomes of control action is an important part of advanced production management. Indeed, farm records are good management tool. These records allow to measure how efficiently resources are used. Farm records are also essential for planning and decision making. Poultry management system is a bioinformatics based intelligent cum expert system. This system was being planned to be an expert system which may guide the broiler and layer farmers regarding day to day management, feeding, biosecurity/disease control decision based mainly on the strain basis as recommended by the management guides or research findings. With adequate effort spent to learn how to use the technologies, they can be used by most farmers in any production area and producing any commodity or mix of commodities. Poultry management system allows the user to manage data on financial, employee, farm building, stock and flock performance aspects.

ABSTRACT INFO

Session: Bioinformatics

New Bioinformatics-Based Discrimination Formulas for Differentiation of Thalassemia Traits from Iron Deficiency Anemia

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Thalassemia traits (TTs) and iron deficiency anemia (IDA) are the most common disorders of hypochromic microcytic anemia (HMA). The present study aimed to differentiate TTs from IDA by analyzing discrimination formulas and provides comprehensive data of hemoglobin disorders prevalent in Pakistan. Among 12 published discrimination formulas, 6 formulas—MI, EF, G&K, RDWI, R, and HHI—were the most reliable to discriminate TTs from IDA. The failure cutoff values were improved by the random forest (RF) decision-tree approach. Moreover, the Shine and Lal (S&L) formula, which completely failed to discriminate IDA from TTs with original cutoff value (<1530), improved with the use of new proposed cutoff value (<1016) and was found to successfully discriminate all cases of TTs from those with IDA.

In addition, 2 newly proposed formulas discriminated TTs from IDA more reliably than the original 12 formulas assessed. The proposed formulas could play a crucial role for clinicians to discriminate between TTs and IDA.

ABSTRACT INFO

Session:
Proteomics

Estimation of Proteolytic Activity of Indigenous Protease Producing bacteria

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Protease is a remarkable group of enzymes that catalyze the breakdown of protein into simpler compound. They have an immense importance in the biochemical reactions as well as in different industrial fields like detergent industry, leather industry, dairy industry, baking industry and pharmaceutical industry .The purpose of present study was to quantify the proteolytic activity of protease producing bacteria. Samples were taken from the poultry shop that included chicken feather samples. Isolated bacteria was characterized morphologically and biochemically. Qualitative analysis of protease activity was checked on Skimmed milk agar where zones of casein hydrolysis indicated positive result. Alkaline protease producing bacterial samples were screened by growing on media supplemented with 7% NaCl and adjusted to pH 9. Optimization of bacterial growth at different pH and temperature was determined. Protease activity (unit/ml) was estimated by quantitative assay for bacterial strains and temperature and pH assay was optimized. DNA was isolated, PCR was performed and sequencing was done for bacteria were found to belong to to Bacillus group. Such indigenous bacteria should be explored more and optimized further for cheaper enzyme production and applications.

ABSTRACT INFO

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Proteomics

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Molecular Mechanisms of Anthracycline Induced Multi Drug Resistance in PC3 and HCT-116 Human Cancer Cells

Shumaila Moazzam* and Alena Sumrin

Chemotherapy is frequently used treatment for hormone refractory prostate cancer but emergence of drug resistance renders it ineffective. Drug combinations are used that do not result in cross resistance or ideally show collateral sensitivity. Here we have shown that epirubicin resistant prostate cancer PC3 cells showed cross resistance to gemcitabine and ara-C in drug sensitivity assays. In comparison HCT-116 that was made resistant to epirubicin in the same way did not result in cross resistance to these nucleoside analogues. Quantitative real time PCR showed up regulation of cytidine deaminase (CDA), ribonucleotide reductase (RR) M1/M2, thymidine kinase 2 (TK2) and multidrug resistance associated protein 1 (ABCC1) in epirubicin resistant PC3 cells compared to their wild type matching cells. Epirubicin resistant HCT116 did not show the gene expression pattern favouring gemcitabine resistance. Protein quantification using Western blotting showed 3 to 25 fold up-regulation of CDA in various epirubicin resistant PC3 lines, whereas HCT-116 being a low CDA expressing line did not exhibit CDA levels high enough to be comparable to PC3. Contrary to previous reports where anthracycline/multi drug resistant cancer cell lines showed gemcitabine sensitivity, we have shown cross resistance in case of prostate cancer cells. Underlying molecular mechanism could be; up regulation of gemcitabine inactivating CDA along with ABCC1, the efflux pump for gemcitabine's inactivated dFdU form. RR M1/M2 and TK2 up-regulation could be the minor contributors in gemcitabine cross resistance. Further research is needed to understand emergence of anthracycline induced cross resistance against nucleoside analogs in different cancer types as these drugs are commonly used together in combination therapies

ABSTRACT INFO

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Protein Engineering of Endoglucanase CelR of Clostridium thermocellum for Enhanced Expression

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Enhanced production and improved properties of cellulases for a greater activity on plant biomass would rank amongst the top priorities for second-generation ethanol production. Protein engineering has emerged as a cutting-edge technology for enhancing enzyme activity and expression level. The present study is aimed at the application of protein engineering technique to the major cellulosomal processive endoglucanase of C. thermocellum, CelR for refining enzyme characteristics through truncation of the native enzyme. The full length native enzyme gene (CelR) and a truncated version without the dockering domains at C-terminus (CelR-CB) were PCR amplified using gene specific primers. The amplified PCR products were T/A cloned in the vector pTZ57 R/T and transformed in E.coli DH5α. The cellulase genes from the confirmed transformed plasmids were sub-cloned in T7 promoter based expression vector pET-28a and expression analysis was done in *E. coli* (DE3) BL21 codon Plus. An SDS PAGE analysis of both the CelR derivatives revealed that the truncated version i.e. CelR-CB showed a two-fold increase in expression level as compared to the full-length enzyme. The increased expression level of CelR in *E. coli* coupled with its increased production therefore makes it a promising method for augmenting the recombinant enzyme production for potential applications.

ABSTRACT INFO

Session: Proteomics

HPLC and gel based decomplexation of Echis carinatus sochureki venom

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Snakes have been considered deadly organisms as their venom can cause severe health hazards and often leads to death of the victim. More than 600 species of snake have been classified as potentially lethal or venomous throughout the world. Among these Echis carinatus, Naja naja, Russell's viper and Bungarus species are commonly found in Pakistan and are responsible for high morbidity particularly in rural areas. Our study presents HPLC and gel based decomplexation of proteins and peptides from the venom of Echis Carinatus sochureki, commonly called saw scaled viper.

Snake venom is modified saliva containing proteins and peptides with fatal effects when injected into the prey. Phospholipases, Serine proteases, metalloproteases, hyaluronidases and L-amino acid oxidases are main venom enzymes which induces cytotoxicity, neurotoxicity, hemorrhage and other life-threatening symptoms. In our study, we performed HPLC based separation and two-dimensional gel electrophoresis for decomplexation of venom components.

Snake venom was collected from different districts of province of Sindh, lyophilized and stored at -20°C. RP-HPLC was performed using C18 column and fractions were collected and subjected to 12% SDS PAGE, stained with coomassie blue. HPLC chromatogram and SDS PAGE results showed a wide range of proteins from low to high molecular mass. Further analysis was conducted by 2D gel electrophoresis, 80 µg of venom was applied on a 7 cm IPG strip with pH gradient of 3-10 pH followed by SDS-PAGE and coomassie staining. The 2D gel showed the presence of acidic to basic range proteins. The combined analysis showed the presence of proteins in the range of 92-22 KDa as a major portion while the high and low molecular mass proteins were found to be in less abundance. The 2D gel also revealed that the low molecular mass proteins are mostly acidic in nature.

ABSTRACT INFO

Session: Proteomics

Large-Scale Proteomics of Jatropha curcas Seeds Unravels the Site of Synthesis of Phorbol Esters

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Jatropha curcas seeds are a potential source of raw material for biodiesel production, but the exploitation of this potential is hampered by the presence of high amounts of phorbol esters (PE), a class toxic diterpenes. Currently, the biosynthetic pathway of PEs is poorly understood, making it difficult to devise genetic strategies for engineering plants which lack these compounds. We performed a large-scale quantitative proteome analysis of the endosperm and inner integument from developing seeds, as well as of plastids isolated from these two tissues, resulting in the identification of more than 5000 proteins. This allowed us to reconstitute enzymatic pathways for the biosynthesis of fatty acids and amino acids, establishing the deposition pattern of seed storage proteins during seed development and identifying a plethora of hydrolases involved the programmed cell death both in the endosperm and seed integument. However, we were unable to identify key enzymes of the biosynthetic pathway related to PE, which lead us to suspect that PE are synthesized in maternal tissues other than the seed integument and translocated to the developing seed. To gather confirmatory evidence for this, we used qPCR analysis to determine the expression pattern of casbene synthase, a key enzyme in the biosynthesis of PE, in roots, leaves, embryo, endosperm, integument and pericarp of developing fruits. Of the 14 putative casbene synthase gene analyzed, nine were expressed only in roots, while one was expressed both in roots and leaves. Together, our proteomic and gene expression analysis provides insights about the synthesis of toxic PE and in addition is a start point for experimental approaches to obtain J. curcas varieties which lack PEs.

ABSTRACT INFO

Session: Molecular Medicine

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Antioxidant potential of Aqueous and methanolic extracts of Cymbopogon citrates (lemon grass) against scavenging radical compounds

Nighat zia ud Den*[1], Muhammad Shahid[2], Amer Jamil[3]

Medicinal plants have a great significance in human health all over the world. Cymbopogon citrates (lemon grass) has long been widely studied with respect to its medicinal importance. Keeping in view the importance of flavonoids and other bioactive components this study focused on assessing biological activities using different extracts at different temperature treatments. The disc dissemination system was utilized to survey the antimicrobial power of the methanolic and aqueous extracts of Cymbopogon citrates. It was observed that all tested microbial strains were sensitive (>0.51 activity index) to aqueous extract except for S. aureus that displayed a higher antimicrobial effect than other microbial species. Similarly, aqueous extract of lemon grass had high total phenolic (0.4µg/mL) and flavonoid contents as compare to methanolic extracts. The present findings show that Cymbopogon citrates aqueous extract at temperature 120 °C had a high biological activities due to temperature treatment effect. The results of this study hint at the potential of Cymbopogon citrates as source of healing and medicinal agent.

ABSTRACT INFO

Session: Molecular Medicine

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Characterization of Vancomycin-Resistant Enterococcus faecium Capsular Polysaccharide Gene Cluster

Liaqat Ali[1, 2]

Vancomycin-resistant Enterococcus faecium has emerged as one of the most common nosocomial pathogen of health-care associated infections that is difficult to treat with available antibiotics. These pathogens produce capsular polysaccharides which play a significant role on the bacterial cell surface, including adhesion, virulence and evasion from phagocytosis. With the goal of identifying new vaccine antigens, we identified a putative Epa like capsule gene cluster in E. faecium, containing 11 glycosyltransferases including capsular polysaccharide biosynthesis protein with homology to the staphylococcal and pneumococcal CapD protein. The mutation of this gene led to reduced growth, a minor phenotypic structural alteration, increased cell surface hydrophobicity and a reduced biofilm formation as well as antibiotic susceptibility compared to wild-type and the complemented mutant. In this study, the purified crude polysaccharides were investigated for the ability of E. faecium capsule to confer resistance to opsonophagocytosis. The antibiotic strains E. faecium U0317 and E0155 were well opsonized in the presence of anti-U0317 antibodies but not in the presence of anti-LTA. Also showed an increased susceptibility of the capD mutant to opsonophagocytic killing in the presence of some polyclonal antibodies. These findings make E. faecium an interesting candidate for the study of novel CPSs as vaccine candidates. The mutant showed a decreased binding to human uroepithelial cells in comparison to the wild-type strain. Further, we functionally characterized the mutant in animal infection models which demonstrated a higher bacterial colonization during bacteremia and a lower virulence in the rat endocarditis and the mouse UTI model, respectively.

ABSTRACT INFO

Session: Biosafety

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Biosafety of Genetically modified microorganisms (GMOs)

Zeshan Zulfiqar*, Farooq Sarwar and Waqas Azeem

Genetically modified microorganisms (GMOs) are prepared through genetic engineering by the insertion of small foreign fragment of DNA carrying the gene of interest in to the local organism (Host). These are the valuable tools for development in food, agriculture, health and other industries. Biosafety regarding GMOs refers to the prevention of unwanted exposure of toxins, chemicals, radiation and harmful biological agents into the environment and on human and animal health. It demonstrates the principles, procedures and strategies to be acquired to establish the environmental and human health safety. Recent advances using GMOs in the field of life sciences have been gaining importance to ensure the public health and environmental safety. One of the major risk related to GMOs is the Horizontal Gene Transfer (HGT) which occurs in response to change in environment. HGT may affect the ecological niche of the host organism and may be a harmful source related to human health and environment. Some transgenic traits introduced through GMOs in host like pesticidal toxin may affect the non-target species as well as crop pest. To ensure the safety concern, Cartagena Protocol on Biosafety (CPB)" has been adopted. It includes the movement of living modified organism by the fixing the area for them in a restricted manner and take steps for risk management. Risks relating to GMOs should be assessed on the basis of scientific data to evaluate the probabilities of possible outcomes. Public access relating to biosafety of GMOs must also be necessary including the labeling of product, food safety standards, and general consumer protection laws to create awareness among public to the commercial promoters of GMOs.

ABSTRACT INFO

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Risk assessment studies of CEMB triple gene cotton fed rats

Qasim Ali*, Ahmad Ali Shahid, Tahir Rehman Samiullah, Abdul Qayyum Rao, Idrees Ahmad Nasir and Tayyab Husnain

Genetically modified (GM) crops possessing the insecticidal and herbicide tolerance traits offer a new strategy for crop protection, however, at the same time rise concerns in term of biosafety. The described study presents the findings of a 90 days safety assurance study with Sprague-Dawley fed cotton expressing the Cry1Ac, Cry2A and CP4 EPSPS genes. There were 30 rats in the study divided into five groups, each group contains 6 rats (3 female, 3 male). Rats in group (I) was fed on normal diet, group (II) and (III) rats were on diet containing 10% and 30% (w/w) of non-transgenic cotton (equal ratio of seeds and leaves) while group (IV) and (V) on 10% and 30% (w/w) of transgenic cotton (equal ratio of seeds and leaves), respectively. During study, all rats in both control and treated groups grew well without any significant difference in their weight gain, food/water intake, and appearance. Similarly, no marked difference was observed in clinical pathology parameters; blood biochemical analysis (hematology, detection of residual target proteins in serum, serum chemistry), organ weight, organ morphology (colour, texture, lesion, necrosis), urinalysis and microscopic examination of organs. In conclusion, study suggests that transgenic cotton harboring the insecticidal and herbicide-resistant genes has no adverse effects on the overall health of Sprague-Dawley rats.

ABSTRACT INFO

Session: Food biotechnology

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Occurrence Of Gram Negative Pathogenic Bacteria In Ready To Eat Leafy Vegetables From Lahore

Zulquernain Haider*[1] and Sikander Sultan[2]

Leafy green vegetables are good source of nutrition, for this reason they are extensively used in variety of food. Due to nutritional contents, leafy greens are prone to be contaminated with bacterial pathogens during cultivation, harvesting and storage. Many outbreaks that has caused a vast morbidity and mortality have been reported yet in which leafy greens were the source of pathogen. For this reason, three extensively used leafy greens coriander, mint and lettuce were selected and sampled form 16 sites from Lahore in 4 groups urban, suburban, suburb and supermarkets. A sum of 75 samples of these three leafy greens were analyzed for aerobic plate count and coliform count. Based on colony morphology and lactose fermentation gram negative bacteria were isolated and purified for identification. Emerging trend of antibiotic resistance was also taken into count and antibiotic resistance was analyzed by Kirby Bauer Method. The APC was ranged in 2.0×107-1.1×109 CFU/g. Coliform count was 6.0×105 to 4.8×107. Sixteen types of gram negative bacteria were identified after purification. All the selected strains were Multiple antibiotic resistant. The highest multi drug resistant were observed in case of Proteus and Weeksella species.

ABSTRACT INFO

Session: Plant Biotechnolgy

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Citrus biotechnology: prospectus and limitations

Ammara Noreen*, Abdul Manan Saleem, Dr. Muhammad Azhar Bashir and Muhammad Ikhlaq

Citrus is among the world's leading fruit crop in terms of area as well as production. There is a wide range of edible and non-edible species of Citrus. Genetic improvement through conventional techniques is a difficult task for plant breeders due to its complex reproductive biology, polyembrony, long juvenile phase and sexual incompatibility. Plant biotechnology offers a reliable solution for efficient citrus varietal improvement. Hence, it is challenge to improve Citrus species using in vitro techniques. As citrus a woody plant, it is prune to several tissue culture issues like browning, translucency etc., yet continuous efforts made it possible to establish well-developed protocols for citrus tissue culture. With the development of tissue culture protocols somatic hybridization, being overcoming self and cross-incompatibility is playing a vital role in inter-specific and inter-generic hybrids development. Another technique that has been developed as an important tool for introducing desirable genes into Citrus species is Agrobacterium mediated transformation. Transgenic against biotic (viruses, bacteria) and abiotic stresses have been developed. On-going citrus research focuses mainly on incorporating resistant genes to improve quality and yield of citrus. This review covers the advancements in citrus improvement, and suggests future directions.

ABSTRACT INFO

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Lead, Cadmium and Zinc phytotoxicity alter DNA methylation levels to confer heavy metal tolerance in wheat

Sarfraz Shafiq*[1,2], Qudsia Zeb[3], Asim Ali[2], Emilie Widemann[4], Rashid Nazir[2]

Being staple diet, wheat nutritionally fulfills all requirements of human health and also serves as a significant link in food chain for the ingestion of pollutant in humans and animals. Therefore, the presence of the heavy metals i.e., Pb and Cd in the soil is not only responsible for the reduction of wheat crop yield but also the potential threat to human and animal health. Efforts have been made to understand the physiological, biochemical and molecular basis of metal stress in wheat; however, epigenetic basis of wheat plant resistance to the heavy metal stresses is not yet investigated. After the phenotypic screen of eight high yielding wheat varieties in response to Pb stress, we further validated the most resistant Pirsabak 2004 and sensitive Fakhr-e-sarhad against the Cd and Zn toxicity through the phenotypic, biochemical and molecular approaches. We found that expression of DNA methyltransferases and DNA methylation levels vary greatly between the Pirsabak 2004 and Fakhr-e-sarhad, thus differently regulates the basal gene expression. Furthermore, in response to Pb, Cd and Zn metal stress, the expression of some TaABCCs and TaHMA2 metal detoxification transporters is upregulated in Pirsabak 2004 compared with control and Fakhr-e-sarhad, which is due to the CG DNA hypomethylation at the promoter of these transporters in Pirsabak 2004. The data indicate that DNA methylation levels in wheat are associated with tolerance against metal toxicity. Our results, therefore, highlight the implication of DNA methylation in metal stress tolerance, further arguing for the importance of epigenetic modifications in future breeding strategies of wheat.

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Genome-Wide Analysis Characterization and Evolution of SUC Genes of upland cotton

Muhammad Abdullah*, Yunpeng Cao, Xi Cheng, Xueqiang Su, Awais Shakoor, Jun Shan Gao and Yongping Cai

In higher plants sucrose is an essential element of life cycle. It is mainly produced by photosynthesis source tissues and is transported to sink tissues, where its consequent cleavage in the sink tissues is the first step for utilization of the photoassimilate for various metabolic pathways. In plant, Sucrose transporters are related to endogenous and exogenous mechanisms of detoxification associated with secondary metabolites such as alkaloids, flavonoids, anthocyanins and other secondary metabolites. However, genome-wide analysis of the GhSUC family is rarely reported in upland cotton. In this study, we use the whole genome survey to analyze the sequence characteristics, phylogenetic evolution and expression patterns of the Sucrose transporter gene family in Gossypium hirsutum L. The total of 20 SUC transporters are identified in the whole genome sequence of upland cotton (Gossypium hirsutum L.), which may be categorized into four subfamilies with possible diverse functions such as transport of proanthocyanidins, xenobiotic compounds, alkaloids, regulation of disease resistance and response to abiotic stresses. Subsequently, the gene structure, evolutionary relationship, physical location, conservative motifs and gene expression pattern of SUC genes have been analyzed in brown cotton. These results will provide a new outlook on upland cotton SUC gene family for their prospective roles in transport of anthocyanin and provide a new abstract for future analyzing the function of SUC genes and improving the pigment quality of colored cotton.

ABSTRACT INFO

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Cytotoxic and Phytochemical activities of Anti-Cancerous Plants *Viscum album* L.and *Korthalsella japonica* (Thunb)

Hina Bangash, Ikram Khan, Zabta Shinwari, Khaista Rehman and Muhammad Ali*

Anti- cancerous plants i.e Viscum album L.and Korthalsella japonica (Thunb) from KP, Kohistan valley (upper Dir) were used for this study. The objective of the current research project is to check the biological and cytotoxic effects of the crude extracts of the medicinal herb. To assess the antioxidative potential of both plants, the highest DPPH radical scavenging activity was exhibited by VA leaves part has the highest value. While assessing reducing power, VA stem extracts of methanol: water were highest of all i.e 67.2 µg/mg of extract as compared to VA leaves. Among cytotoxicity assays, protein kinase inhibition assay against Streptomyces fungi revealed that chloroform stem extract showed the highest activity in all stem extracts with above 20 mm diameter clear zone indicated cytotoxicity. In brine shrimp cytotoxic lethality assay, the highest inhibitions was recorded at the concentration of 1000 µg/ml in which n-hexane : ethyl acetate extract of leaves manifested highest cytotoxicity where 37 µg was the lethal dose to kill shrimps up to 50 %. In case of antileishmanial activity, both the leaves and stem extracts exhibited high cytotoxicity on the whole against promastigote and amastigote form of leishmanial parasite but promastigote results was much promising. Our results show that both of the plants Viscum album L.and Korthalsella japonica (Thunb) have the potential to discover potent anticancer agents. Bridging the precious cytotoxic compounds in with the cutting-edge technologies and to focus on their mode of actions would be a milestone in developing anticancer therapies.

ABSTRACT INFO

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Cotton Leaf Curl Virus (CLCuV) association with leaf epicuticular wax load, composition and whitefly population on different cotton varieties

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Experiment was conducted on existing cotton varieties against Cotton Leaf Curl Virus (CLCuV) and whitefly infection. The objective of this study was to determine the difference in leaf epicuticular wax load and composition of CLCuV resistant and susceptible cotton varieties. Settling behaviour of whitefly in previous studies showed more whitefly population on CLCuV susceptible whereas less on CLCuV resistant cotton plants. Correlation analysis of CLCuV infection with whitefly population and leaf epicuticular wax load was performed. CLCuV infection has significant positive correlation with whitefly population and significant negative correlation with leaf epicuticular wax load. These results depicted more leaf epicuticular wax load and less whitefly attack on G. arboreum as compared to G. hirsutum varieties. It was also hypothesized that resistant cotton varieties may have different wax composition than susceptible varieties. For this purpose, GC-MS analysis of leaf epicuticular wax of both types of varieties was performed. GC-MS results of CLCuV resistant and susceptible cotton plants also showed differences in quantity and composition of leaf epicuticular wax. Based on these results, we can conclude that leaf epicuticular wax acts as a barrier against CLCuV and whitefly infection. Different biochemical compounds present in leaf epicuticular wax along with their quantity, are responsible for the resistance and susceptibility of G. arboreum and G. hirsutum varieties.

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Insect bioassay to verify the tolerance level of Helicoverpa armigera on Bt cotton genotypes containing Cry1Ac gene

Shahid Nazir*[1], Muhammad Zaffar Iqbal[1] and Dilbar Hussain[2]

Helicoverpa armigera is one of the most devastating insects of cotton which severely affects the yield globally. Various crop protection approaches including transgenic technology have been used to cure the yield against this insect. Introduction of bacterial Cry1Ac gene have modernize the cotton crop with effective insect control and protect the yield very efficiently. Now it is noticed that this insect being survived on Bt cotton and damaging the crop. Hence, a study was designed to verify the insect tolerance level against Cry1Ac toxic protein produced in bollgard cotton genotypes. Presence of Cry1Ac gene was confirmed by conventional PCR using genomic DNA as template and toxin accumulation levels were investigated by quantitative ELISA. Laboratory reared larvae of H. armigera were released under controlled conditions on detached young leaves with variable toxin levels ranging from 0.24-3.72 µg/g on fresh weight basis. 10 larvae/leaf were allowed to feed and insect survival data was recorded on daily basis. Maximum survival rate was found on leaves containing 2.11 µg/g of toxin, while above this level no larvae was survived. Data was recorded in triplicate fashion upto twelve days. These results showed that this Cry1Ac gene being losing its control on insect and is alarming situation for cotton improvement. Furthermore, it could also be possible that insect may got some mutations in their genome and tolerate the toxic protein which require further study. Hence, it is concluded that new versions of insect resistant genes should be introduced in cotton for effective insect control in future.

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An overview of wheat Biotechnology at Biological Sciences Department of FCCU

Asma Maqbool*, Muhammad Irfan, Aftab Bashir and Samreen Mohsin*

Agricultural biotechnology is the need of the hour in order to meet the challenges of food security. With well-established labs, Department of Biological Sciences at Forman Christian College has been focusing on wheat biotechnology research as wheat is the staple food of Pakistan. To establish wheat transformation, Pakistani wheat varieties were screened for Agrobacterium-mediated transformation, tissue culture response, phytic acid content, phytase activity as well as iron and zinc content. Further, we are trying to improve wheat varieties with different aspects like enhancing nutritional content and fertilizer use-efficiency. For improving nutritional content, transgenic wheat has been developed with enhanced phytase expression in endosperm that will result in reduced phytic acid content and an increase in iron and zinc content. This transgenic wheat has been evaluated till T3 generation and after handing over the seeds to Ayub Agricultural Research Institute, this project has been successfully completed. Secondly, phosphorus uptake by wheat is also being improved by enhancing extracellular phytase secretion from roots which will enable the transgenic plants to use organic phosphorus (phytate). This will not only be beneficial for wheat but also have a positive effect on the soil system. The transgenic wheat has been tested till T2 generation uptill now and three events have been selected for field trials. Moreover, nitrogen and phosphorus fertilizer use efficiency is also being enhanced in wheat via expression of Dof1 and PTF1 transcription factors in wheat varieties, respectively. Construct for both transgenic wheat have been developed and T1 plants have been obtained. Drought and salt tolerant transgenic wheat is also being developed but this work is at initial stages.

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Establishment of Ethanol Inducible System and Protein Analysis of LTB-L1 Expressing Transplastomic Nicotiana tabacum Plants

Kehkshan Gull, Iqra Yunas, Bushra Mirza and Mohammad Tahir Waheed*

Previously, constitutive expressions of LTB-L1 lead to pleiotropic effects like chlorosis, stunted growth and sterility on transplastomic tobacco plants. It was hypothesized that these deleterious effects on plants were due to the intrusion of LTB-L1 gene or its product. The current study was done to verify this hypothesis and to check the maximum protein expression using inducible expression system. In the current study, tobacco plants containing LTB-L1 gene under the control of inducible promoter were used. Plants were sprayed with 5% ethanol to induce expression of LTB-L1. As soon as the plants were sprayed, leaves started showing pale patches, which gradually increased and finally leaves almost completely bleached out at the end of spraying period. Wild type plants showed no such effects, proving that these effects were only due to the expression of LTB-L1. Homoplasmic state of transplastomic plants was confirmed by RT-PCR and seed germination efficiency on selection media. Presence of proteins after induction was confirmed by coomassie staining and protein of interest was quantified by western blotting. The maximum protein content observed was 7.2% of total soluble protein at day 11 of induction. Further structural confirmation analysis of LTB-L1 protein was done by antigen capture ELISA. It is concluded that observed pleiotropic effects on plants were due to LTB-L1 expression. Hence in future, it is recommended to use inducible system for the expression of transgenes in chloroplasts for a tightly controlled expression of proteins. In future, this inducible expression method can be established for other plant species, especially edible crops such as lettuce and broccoli.

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Revealing antifungal potential of Trichoderma harzianum derived chitinase gene

Iqra Yousaf, Idrees Ahmad Nasir* and Bushara Tabassum*

Agricultural crops feeding more than half of the population of Pakistan is threatened by various fungal and bacterial pathogens resulting in significant yield losses. Contemporary approaches to overcome these diseases include the use of chemicals, biological methods and genetic engineering. Genetic engineering through introduction of antifungal gene in plants brings resistance towards specified pathogen. Trichoderma, a potential biocontrol fungus has the ability to breakdown chitin, an essential biopolymer of fungal cell wall. This is achieved through chitinase enzyme. In the current study, full length coding sequence of chitinase gene was amplified and characterised. The deduced sequence submitted in GenBank data base under accession # KY290959. Bioinformatic analysis predicted acidic, hydrophobic, thermostable and soluble nature of chitinase protein. Further, the 35 KDa recombinant protein of chitinase was expressed in *E. coli* strain BL12 under T7 promoter. The expression of 35 KDa recombinant chitinase protein was observed in SDS-PAGE and confirmed with His tag specific antibodies through western blot. A single purified fraction of recombinant chitinase protein was obtained. Upon chitinase activity assay it was found that protein has 6.2 U/ml of endo chitinase enzyme. Strong antifungal potential against C. falcatum and F. oxysporum was also observed in in-vitro fungal inhibition assay.

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E.coli Expression of HA gene of H9N2 Influenza Virus

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The H9N2 strain of avian influenza virus has become the greatest threat because it is one of the major causes of economic losses in the poultry industry. This situation has enhanced the struggles for preventive approaches. The current study is the one of the determined attempt in this regard. The present study is the part of edible vaccine production against avian influenza caused by H9N2 viral strain, through expression and characterization of the immunogenic gene of Influenza H9N2 strain. Sequence of Hemagglutinin (HA), an immunogenic gene of H9N2 Influenza virus strain was taken from NCBI, It was commercially synthesized after codon optimization according to Maize Zea Maysand supplied in pUC57 cloning vector. The pUC57 containing HA gene was verified through amplification of 1683bp amplified PCR product obtained by using gene specific primers, visualized on 0.8% Agarose gel after its isolation as plasmid from *E. coli*strain Top 10. The PCR amplified product was ligated into a TA cloning vector (pTZ57/RT). The separation of 1683bp fragment fromthe pTZ57/RT through restriction digestion with SalI and XhoI confirmed its successful ligation into TA vector. For expression of HA gene, it was ligated into pET30a expression vector and transformed into E.coli Rosetta cells, an expression host. The induction of expression was also done by adding 100mM IPTG in a growing culture for higher yield followed by categorization of the protein on the basis of sizes on SDS polyacrylamide gel. The appearance of 67k Daprotein on SDS PAGE confirmed the successful expression of HA protein in *E. coli*. Furthermore the specificity of HA protein was also confirmed through antibody antigen reaction on nitrocellulose membrane (Western Blotting).

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An In-Silico Overview: Role of F3'5'H in color alteration in Cotton

Ammara Ahad*, Sana Tahir, Ramlah Ejaz, Rabia Nawaz, Sala Hud Din, Sidra Akhtar, Saira Azam, Ambreen Gul, Mukhtar Ahmed, Aysha Latif, Abdul Q Rao, Ahmad A Shahid and Tayyab Hasnain

Anthocyanins are water-soluble pigments giving the purple, red and blue colors to various flowers and fruits. Anthocyanins are widespread amongst seed plants associated with physiological roles in plants, to attract pollinators, seed dispersers and against UV–B protection. A key enzyme of this flavonoid pathway, Flavonoid 3', 5' hydoxylase, F3'5'H is involved in delphinidin production. Delphinidin gives blue or violet hues to flowers and fruits naturally. Molecular level modifications in pigmentation pattern of cotton fiber, is an attractive and innovative area of research. In present study, comparison of F3'5'H in Gossypium hirsutum (Cotton) and Viola × wittrockiana, sequence alignment for homology, protein modeling along their structural validation and molecular docking analysis were computed. Docking studies were carried out using AutodockVina and the ligands used were Naringenin and Quercetin. Analysis of results showed the best binding energy values of Viola F3'5'H i-e -8.9 & -9.9 with both ligands respectively. In vitro Anthocyanin quantification assay also showed enhancement of these secondary metabolites in transgenic plants. Maximum anthocyanin concentration was found to be 1.79 µg/g as compared to non-transgenic control which was 0.86 µg/g. Based on these bioinformatics as well as quantification assay results it was hypothesized that besides the presence of this gene in cotton naturally, an over expression of Viola F3'5'H had a potency to alter pigmentation pattern in cotton fibers. Stronger expression of transgene would lead to immense pigmentation as a result altered color in fibers would be achieved.

ABSTRACT INFO

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Studies on Antifungal Potential of Ganoderma lucidum against Alternaria alternata

Muhammad Asif*, Muhammad Ali, Nasir Ahmad, Ahmad Ali Shahid and Muhammad Saleem Haider

The present research was focused to study the antifungal potential of *Ganoderma lucidum* proteins against *Alterneria alternata*. Proteins were extracted in 50 Mm sodium phosphate buffer by macerating powdered fruiting body of G. lucidum. Extracted protein solution (crude extract) was filtered and tested for antifungal activity by spreading different volumes (500 µL, 1000 µL, 1500 µL and 2000 µL) of extract on Malt Extract Agar plate that were subsequently inoculated with spore suspension of A. alternate. Results showed that 100% fungal inhibition was achieved on plates containing 2000 µL of G. lucidum protein extract, however only 38% inhibition was observed in case of 50 µL of protein extract. Partial purification of antifungal proteins was done by fractional ammonium sulfate precipitation (20%, 40%, 60% and 80% Ammonium sulfate saturation). Higher level of antifungal activities was depicted by the proteins precipitated at 20% and 60% Ammonium sulfate precipitation. Further, purification of antifungal proteins through chromatographic and electrophoretic methods is in progress.

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Pseudomonas sp. AF-54 exhibits multiple traits of plant growth promotion on *Helianthus annuus* L. crop of Azad Jammu and Kashmir

Afshan Majeed*[1], M.Kaleem Abbasi[1], Sohail Hameed[2], Asma Imran[3], Tahir Naqqash[4], Kashif Hanif[5] and Shaista Javaid[6]

The use of plant growth promoting rhizobacteria is a promising strategy for sustainable agriculture production. The aims of the present study were to isolate, characterize and identify sunflower associated beneficial bacteria and to evaluate their inoculation and colonization potential. The soil sample was taken from Diyar Gali, a completely unexplored area from Himalayan Mountain region of Dhirkot (subdivision), Azad Jammu and Kashmir. A putative rhizospheric bacteria was isolated from sunflower rhizosphere and identified as Pseudomonas sp. AF-54 through 16s rRNA analysis. The strain solubilized insoluble phosphorus (P) up to 48.80 µgmL−1 and caused a decreased in pH (3.04). The HPLC analysis showed 23.9 µgmL-1 indole-3-acetic acid, acetic acid, malic acid and gluconic acid production. While, gas chromatography showed nitrogenase activity up to 44.28 nmoles mg-1 protein h-1. AF-54 metabolized 79 out of 96 carbon sources and showed biocontrol activity against Fusarium oxysporum also found resistant to a number of antibiotics. Upon inoculation in soil-free medium (growth pouches), in sterilized soil (pots) and in field under natural conditions at two locations i.e., Rawalakot, AJK, and Faisalabad, Pakistan, AF-54 was found an effective and potent strain in augmenting sunflower growth, yield and oil contents and NP uptake compared with 50% N and P fertilizers treatments. AF-54 showed strong association with sunflower roots up to 45 days in population dynamic studies and the colonization potential was confirmed through a series of high throughput microscopy techniques including yfp-labelling, fluorescent in situ hybridization (FISH) and biofilm production techniques coupled with confocal laser scanning microscopy, in both sterilized and natural conditions. Based on the results of this study, it is concluded that the potential PGPR strains named Pseudomonas sp. strain AF-54 can be used as biofertilizer for sunflower crop for enhancing yield and to minimize the use of chemical (NP) fertilizers.

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Cloning, Characterization and Expression Studies of Argonaute gene from Barley

Muhammad Umar Bhatti* and Nida Toufiq

Argonaute protein family members play a key role in the gene regulation and are guided by small-RNA. Short interfering RNAs (siRNAs) steer these proteins to target m-RNA molecules for silencing or suppression. Very less information is available on Argonaute proteins and their expression. This study was carried out to clone and characterize Argonaute gene in barley and also for significant protein expression studies. Fresh leaves of Barley were used to isolate the DNA. Amplification of the desired fragment was confirmed by repeated PCR analyses at the elongation temperature 61.0 0C. After successful amplification, Argonaute gene was ligated in TA cloning vector and transformed in cloning host Escherichia coli (dh5α) competent cells using heat-shock method. Inserted gene in E.coli cells was confirmed by PCR, restriction digestion and sequencing. Sequence obtained was analyzed by Basic Local Alignment Search Tool (BLAST), and it showed 99.8% homology with the reported gene. Subsequently, argonaute gene was cloned in *E. coli* expression vector pET30a for protein expression. E.coli expression host, Rosetta, was chosen for protein expression because of its efficiency to express proteins. Transformation in Rosetta was accomplished by heat shock method and positive colonies were confirmed, for the construct, by PCR and restriction-digestion. Positive cultures were then induced with different concentrations of IPTG for Protein expression. Estimated size of the protein was 62.8 kDa, which was confirmed using SDS-PAGE. Western Blotting technique was applied for further expression studies. After repeated experiments, the Histag polyclonal antibodies successfully detected the presence of approximately 62.8 kDa band of Argonaute protein. Characterization of Argonaute gene in barley will help us to analyze the genetic diversity of this gene and to observe its expression closely, so as to benefit from its role as major protein in RNA- silencing.

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Identification of Date Palm (Phoenix dactylifera L.) Cultivars through Single Nucleotide Polymorphism (SNP) Makers

Nadia Faqir*[1], Aish Muhammad[2], Ghulam Muhammad Ali[2], Armghan Shehzad[2], Hafeez ur Rahman[3] and Muhammad Zeeshan Hyder[4]

Date palm is an important fruit crop of Pakistan. It is monocotyledonous, dioecious plant of Arecaceae family. Female trees are of interest to farmers for their fruit. To develop some genetic identification system for identification of date palm cultivars more than 3.5 Kb of DNA fraogments belonging to different chloroplast genes were sequenced. All these sequences were near identical. Further twelve DNA fragments, that were reported to have single nucleotide polymorphism, were sequenced. Analysis of these SNPs indicated three fragments have the highest marker index (MI) of 4.61, 3.61 and 2.26 and bear eight, seven and five SNPs respectively. Using this data, an SNP typing system was developed for varietal identification of date palm cultivars. The system can distinguish not only all the seven studied cultivars from Pakistan but also other cultivars from the world through unique SNPs signatures. To test this system further, four highest SNPs bearing fragments were sequenced from 30 more varieties from Pakistan. The data of SNPs from these four fragments were able to resolve 70% of the varieties with unique signatures. The study suggests that SNPs might be important markers to study closely related cultivars, and in some instances, might prove superior even to sequencing of genes. Further, the strategy we employed to study SNPs in date palm, could be expanded to search more SNPs sites to achieve absolute resolution and to identify cultivars and germplasm found in Pakistan and elsewhere in the world.

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Effect of various hormonal regimes on European and local varieties of grapes (*Vitis vinifera* L.) from Pakistan

Naila Ali* and Humera Afrasiab

Hard wood cuttings are used as conventional method of vegetative propagation of grapevine cultivars. However, there are number of factors which influence its vegetative propagation. Those factors may be land, availability of new stock, water and season also; which reduces the chances of new vines. In vitro propagation or tissue culture technology is used to propagate the plants under controlled conditions in the laboratory. This results disease free and healthy plants irrespective to the season. The purpose of our study is to evaluate the effect of different growth hormones on in vitro propagation of different varieties collected from different areas of Pakistan. ong the seven varieties used for this study, four (red globe, autumn royal, crimson and sultania) were European and the remaining three (sundarkhani, perlette and king's ruby) were local. Sultania exhibited the highest shoot induction rate (100% with 4 shoots/culture) on MS medium supplemented with 1.5mg/l BAP and 0.5mg/l BAP+1.0mg/l kinetin after 29 days of inoculation. Highest rate of shoot induction of sultania was followed by perlette (97% shoot induction with 3shoots/culture) on MS medium supplemented with 0.5mg/l kinetin+0.5 mg/l BAP after 28 days of inoculation. The MS medium containing 2.5mg/l IBA was proved best for all in vitro induced shoots, yielding 76% root formation. The hardening of the best proliferated in vitro raised plants of all cultivars was done on soil+sand (1:1).

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Replacement of chemical fertilizers by beneficial microorganisms isolated from bovine manure to be used as biofertilizer.

Dalaq Aiysha* and Zakia Latif*

Plant growth promotion is a hallmark of plant growth promoting bacteria (PGPB). Along with the distinguished property for great potential of plant growth promoting bacteria, they increase the availability of bio-nutrients for the plants. The above mentioned potential of microorganisms is due to the diversified nature of PGPB. These bacteria indirectly changes microbial balance in rhizosphere either by producing beneficial hormones or suppressing a broadspectrum of plant disease agents. The bacterial strains isolated from raw bovine manure have potentials of producing different metabolites. Randomly selected samples were screened out for the isolation of beneficial strains. Selected bacterial strains were subjected to biochemical tests and identified as; Bacillus, Staphylococcus, Streptococcus, Micrococcus, Corynebacterium and Pseudomonas, predominantly. These bacteria were analyzed for plant growth promoting parameters by performing IAA production and nitrogen fixation. Production of beneficial metabolites was also checked by specific media. In conclusion, the selected bacterial strains which exhibit multiple beneficial characteristics could be used as biofertilizer for sustainable agriculture as well as to replace artificial chemical fertilizers increasing environmental pollution day by day to save the environment.

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Basic helix loop helix transcription factors control the jasmonic acid responsive expression of AP2/ERF domain transcription factor ORA47

Muhammad Khurshid[1] and Johan Memelink*[2]

Upon herbivore or pathogen attack plants produce the jasmonate (JAs) hormones. This leads to degradation of JAZ repressor proteins thereby activating transcription factors including MYC2, MYC3 and MYC4, which sets in motion defense gene expression programmes. JAs signaling also induces all known JAs biosynthesis genes in what is considered a positive feedback loop. Overexpression of the AP2/ERF-domain transcription factor ORA47 leads to elevated expression of all JAs biosynthesis genes and to elevated levels of JAs, indicating that ORA47 controls the positive feedback loop. ORA47 is itself a JAs-responsive gene. The aim of this study was to identify the transcription factor(s) responsible for JAs-responsive expression of the ORA47 gene. Based on literature data we explored the hypothesis that ORA47 is regulated by the functionally redundant JAs-responsive transcription factors MYC2, MYC3 and MYC4. The results show that the MYC proteins can bind to a single G-box in the ORA47 promoter. Triple knockout of the MYC genes or overexpression of a stable JAZ1 derivative abolished JA-responsive ORA47 expression, demonstrating the crucial role of the MYC-JAZ module in regulation of ORA47 expression.

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Transformation of Bacterial Cellulose Synthase genes in cotton to improve fiber quality

Sidra Akhtar*, Ahmad Ali Shahid, Abdul Qayyum Rao, Mukhtar Ahmad, Ambreen Gul, Ammara Ahad, Salah Ud Din and Tayyab Husnain

The textile industry is faced with hitches in terms of cost of production and requirements for high quality in the product. Among the fiber quality parameters, fiber strength, fiber length, and fiber fineness are the primary quality properties that influence textile processing. Microbial cellulose differs from plant cellulose with respect to its high crystallinity and purity, high water-absorption capacity, and mechanical strength. As the major component of cotton fiber is cellulose therefore introduction of bacterial cellulose synthase (Bcs) gene into cotton fibers can bring revolutionary changes in the quality of cotton fiber. This study is aimed at expression of bacterial cellulose synthase genes in cotton fiber under fiber specific promoter in order to enhance the fiber length and fiber strength of the targeted cotton variety via Agrobacterium mediated transformation. Bacterial cellulose synthase genes were cloned in pCAMBIA 1301 vector under fiber specific promoter and successful cloning was confirmed by restriction digestion analysis. Agrobacterium mediated transformation of the gene construct into non-transgenic cotton variety was done. As a result of transformation, obtained transgenic plants were shifted to selection free medium in test tubes and were acclimatized afterwards when the plants were ready to be shifted to soil. Molecular confirmation of genes in the transgenic plants is in process which will further confirm the successful integration and expression of the transformed genes leading towards improvement of fiber characteristics at mature stage of the transgenic plants under field conditions. Fiber qualities of local cotton cultivars can be improved by transforming with bacterial cellulose synthase genes. This would ultimately lead to the enhancement of the cotton fiber quality and hence would reduce the import of high quality cotton fiber by fulfilling the desired level of the fiber quality for the Pakistan Textile industry.

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An Inhibitive Enzyme Assay for Detection of Heavy Metals Using Crude Proteases from Fennel, Parsley and Lemongrass

Hira Nasir*[1], Nayyab Nadeem[1], Amber Shahzadi*[1], Mohsin Ahmad Khan[2] and Nadeem Ahmed[2]

The present study was carried out to investigate inhibition of proteases extracted from the leaves of fennel, parsley and lemongrass in the presence of selected heavy metals. The activity in decreasing order for the three samples, lemongrass, fennel and parsley was 0.341 units/ml, 0.330 units/ml and 0.176 units/ml. The heavy metals selected were Fe, Cu, Cr, Co, Ag and Hg. Standard stock solutions for these heavy metals were prepared and protease inhibition studies were carried out using the principle of casein-Coomassie dye-binding. The greatest inhibition occurred on lemongrass protease and both Hg and Co had reduced activity considerably at minimum concentrations of 40 mg/L and 30 mg/L to 66.3% and 64% respectively. EDTA increased lemongrass protease activity to 0.458 and 0.602 units/ml at 10 mM and 1 mM concentrations. Optimization of protease inhibition studies for 30 mg/L of Co and 40 mg/L of Hg was done; and then sensitivity of the enzyme to detect these heavy metals in wastewater samples was evaluated at 37oC (60 minutes incubation time) for cobalt and at 35oC (20 minutes incubation time) for mercury. The crude lemongrass protease could detect Co and Hg at minimum concentrations of 7.21 mg/L and 2.35 mg/L, respectively in environmental samples. These values were also assayed using AAS for confirmation. The data analysis showed that lemongrass protease can be used as a potential biomonitoring tool for detecting elevated toxicity levels of mercury and cobalt in wastewater samples.

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EFFICACY OF GRASS SPECIES TO REMOVE PHOSPHATE FROM WATER SAMPLES

Sana Khalid Moeed*

The main purpose was to investigate the performance of Grass Species (Bermuda grass, Cymbopogon jwarancusa, Crab Grasses) with Vertical subsurface flow, using plants and gravel as medium, in removal of phosphate (P) from agricultural water. Plants selected, were on the basis of their potential for P removal, investigated in earlier studies. A laboratory-scale constructed wetland system (CWS) employing vertical subsurface flow was set up at house scale, with different types of grasses and its capacity for simultaneous phosphate removal and other parameters e.g EC, pH, from a synthetic agricultural water/waste was monitored over a period of three months. The system showed an extremely high P removal of 64% over the whole period of observation. pH, EC and temperature did not differ significantly. Plants started to die due to the extreme weather in the mid period of the experimentation. Then plants showed excellent growth, with good root and rhizome development, and showed potential for phosphate removal. It was concluded that the Constructed wetlands using plants that have capacity to absorb organic matter can be very helpful in the secondary and tertiary treatment of agricultural water.

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Expression of cell wall loosening gene in cotton under fibre specific and constitutive promoters

Amina Yaqoob*, Ahmad Ali Shahid, Mukhtar Ahmad, Mujahid Hussain, Mariyam Maqsood and Abdul Qayyum

Cotton fibre is naturally occurring pure cellulose that plays an important role in the textile industry. In addition to traditional breeding, genetic modifications can improve fibre quality. Many multigenic traits and genes involved in various developmental stages of cotton fibre have been identified in wild plants, including Calotropis procera, which despite its different evolutionary origin shares some fibre traits with cotton. In order to obtain better fibre strength and micronaire values, Calotropis procera genes can be used in the genetic modifications of crop plants such as cotton (Gossypium hirsutum). Since Calotropis spp. are well known for the production of Bast fibres. One of the cell wall loosening genes will be taken from Calotropis procera and transformed into local cotton (*Gossypium hirsutum*) using Agrobacterium tumefaciens. The integration and expression of the Calotropis procera fibre gene in cotton plants will be confirmed by Molecular analysis. The present study will be helpful for the improvement of cotton fibre quality and eventually textile industries of Pakistan.

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Over- expression of chitinase I gene in E.*coli* enhances resistance against fungal phyto-pathogens

Nida Toufiq, Bushra Tabassum* and Umar Bhatti

Agricultural crops undergoes significant yield losses due to certain fungal and bacterial diseases. The identification and characterization of antifungal genes like chitinases can reduce fungal growth, hence tolerance against fungal pathogens is increased. In the present study, chitinase I gene from genomic DNA of Barley (*Hordeum vulgare* L.) cultivar, Haider-93 was isolated. Specific primers were used to amplify ~935bp full length chitinase I gene using DNA as template. Sequence homology of the deduced sequence was analyzed. Barley chitinase class I share 93 % amino acid sequence homology with class II of wheat, whereas, barley class I chitinase and class II chitinase don't share any sequence homology. Further, amplified fragment was cloned in pET 30a vector under the control of T7 promoter using E.coli Rosetta strain. Expression of the recombinant chitinase protein of 35kDa was shown which was highest at 0.5mM concentration of IPTG. Western blot for recombinant chitinase protein of 35kDa was developed with His tag specific antibodies after purifying it with affinity chromatography. These purified recombinant proteins significantly showed antifungal activity inhibiting the growth of certain phyto-pathogenic fungi like Alternaria solani, Fusarium spp, Rhizoctonia solani and verticillium dahliae at concentrations of 80 µg and 200 µg as compared to control.

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Genetic Transformation of Modified Combination of Bt and Plant Lectin Genes to Control major Cotton Pests

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Vegetative insecticidal proteins (VIPs) from Bacillus thuringiensis is considered to be the best possible way to avoid the limitations of resistance build up in insects against the first generation Bt insecticidal crystal proteins (ICPs). Sucking insects are the second most destructive group of insects invading cotton crop by sucking the phloem sap and transmitting viral diseases resulting in loss of almost 2 million bales each year. No report showing a strategy to control of sucking and chewing insects altogether has been documented in recent literature. Use of Vip3Aa in combination with ASAL; a mannose binding lectin gene from Allium sativum was found to have insecticidal activity against both groups of insects when evaluated through Bioinformatics tools. Codon optimized, chemically synthesized Vip3Aa was cloned under Camv35S promoter while ASAL was cloned under RTBV phloem specific promoter. The 4.8 kb cassette of Vip3Aa+ASAL was cloned into plant expression vector pCAMBIA 1301 with the help of XhoI and HindIII restriction sites. A total of ~8000 germinating embryos of cotton were subjected to transformation using Agrobacterium mediated shoot apex method. Out of fifty one putative transgenic cotton plants obtained after selection, eighteen were confirmed through PCR amplification by using gene specific primers. Expression of the transgenes was also confirmed through ELISA and real-time PCR up to T2 generation. Fluorescence in situ hybridization (FISH) will be done to confirm the copy number and location of the transgenes on chromosomes. The current study is aimed to get a transgenic cotton plant that could be resistant to major chewing and sucking insects.

ABSTRACT INFO

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Increased Resistance in Cotton against Sucking Insects through Genetic Modification of Amino acid pathway

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Cotton crop is more susceptible to insect attack and due to heavy sucking insect infestations, the continuous nutrient loss severely affects the growth and yield of cotton. Whiteflies impart three-dimensional damage to the cotton i.e. nutrient deprivation from phloem, sooty mold development and finally the cotton leaf curl virus (ClCuV) transfer. There is need to develop in-built tolerance in the cotton against sucking insects. Targeting the amino acid content of cotton and their overexpression can enhance the resistance of plants to the invading insects. In this study, two organelle specific isoforms of aspartate aminotransferase genes from Oryza sativa were used which are chloroplastic and cytoplasmic specific. Both genes were cloned and overexpressed in cotton under CaMV35S promoter through Agrobacterium-mediated transformation. Both gene cassettes were designed in such a way to clone the genes individually and combine them in one cassette. Putative transgenic cotton plants were acclimatized to field conditions in the tunnel. First screening for T0-transgenic plants were conducted through PCR from genomic DNA of cotton plants. The PCR positive plants were then confirmed for the gene expression through real-time quantitative PCR. Relative expression of mRNAs of AAT genes was determined to be up to 6-fold higher in comparison to non-transgenic cotton plants. This study successfully demonstrated the relative expression of cytoplasmic-AAT and chloroplastic-AAT from Oryza sativa in cotton plants.

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Herbicide Tolerant Triple-Gene Insecticidal KALGIN Cotton

Sarfraz Kiani*, Wajeeha Batool and Tayyab Husnain

Insect pests are a major problem for cotton crop around the world. Cotton in Pakistan is mainly damaged by a number of lepidopteran larvae like spotted bollworms, American bollworms, pink bollworms and Armyworms, etc. These larvae feed on cotton bolls or flowers, and therefore cause great yield losses. Cotton crop is also forced to compete for sunlight and nutrients with unwarranted weeds that often leads to substantial yield losses. Conventional soil-tilling technique is effective to remove weeds but needs intensive labor, time and money. Farmers also spray selective herbicides, which are not effective at removing all types of weeds. In order to address both problems, that is, insect and weeds, we transformed cotton (Gossypium hirsutum L.) cultivars FBS202, FBS203 and FBS906 with three genes (two Bt genes Cry2Ab and VIP3A, and one herbicide-tolerant EPSPS gene) stacked in the same T-DNA using Agrobacterium-mediated embryo shoot-tip method. Stacked two-gene higher expression of Cry2Ab and VIP3A was envisaged to overcome insect-resistance against single Bt-gene. After segregation, two lines in each cultivar giving higher transgene expression in chloroplast were selected for further propagation. Transgene integration was confirmed by Southern blot, while chloroplast-targeted expression was established through confocal microscopy. Laboratory and field bioassays revealed tolerance against first instar larvae of spotted bollworm, American bollworm, pink bollworm and Armyworm. Glyphosate field spray assays (1.6L/ hectare) showed tolerance of transgenic cotton, though all weeds were killed.

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Evaluating drought tolerance in Somaclonal variants of sugarcane (*Saccharum officinarum* L.)

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Drought is a major environmental stress that is resulting in the yield losses of many crops. Sugarcane is basically a water loving crop and scarcity of water is not only effecting its overall production but also its total sugar contents. CPF-248, a drought sensitive variety was used to induce stress tolerance using somaclonal variants. Various combinations of callogenesis and regeneration media were used to induce soma clonal variations. The plants thus achieved were subjected to drought selection pressure using different concentrations of PEG, the selected plants were referred as in vitro selected putative somaclonal variants (IPSV1 and IPSV2). These two plants were than subjected to biochemical analyses for their comparison with the parent plant. The activity of antioxidants activity like SOD, APX, CAT, POD, ascorbate and carotenoid was analyzed and found to be more in IPSV1 and IPSV2 as compare to the parent genotype. The chlorophyll a and b contents were also found to be more in IPSV1 than IPSV2 than parent genotype. Proline contents and free amino acids accumulation as osmoprotectants observed to be more in IPSV2. Increased activities of antioxidants and high accumulation of osmoprotectants as compare to parent genotype are the indicators of more stress tolerance capabilities of the In Vitro selected putative somaclonal variants over parent genotype. The experiment is a guideline for the future researchers to prefer somaclonal variant approach to produce stress tolerant plants over traditional methods of breeding.

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Functional modulation of drought tolerance in microtransgenic barley

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Drought is the most serious abiotic stress, which causes crop loss worldwide. Here we identified a novel microRNA (designated miRX) of 21 nucleotides (nt) in barley. Its precursor (designated pre-miRX) and primary transcript (designated pri-miRX) were also identified, whose lengths are 73 nt and 559 nt, respectively. The identified upstream sequence of pri-miRNA contains both the TATA box and the CAAT box, which are both required for transcription initiation. Transient promoter activation assays showed that the core promoter region of pri-miRX ranged 500 nt from the transcription start site. In transgenic barley over-expressing wheat DREB3 transcription factor, miRX was highly expressed compared to the same miRNA in non-transgenic barley. However, the high expression was not directly controlled by the wheat DREB3 transcription factor. Genomic analysis revealed that the miRX gene was a single copy located at the short arm of chromosome 2 and only conserved in Triticaceae, but not in other plant species. Notably, transgenic barley overexpressing miRX showed significant drought tolerance. Degradome library analysis and other tests showed that miRX targeted many genes including transcription factors via the cleavage mode. Our data open an excellent opportunity to develop drought stress tolerant crops with miRX in the future.

ABSTRACT INFO

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Genetic Modification of Cotton for Improvement of Fiber Characteristics

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Cotton being the premium source of natural fiber faces the problem of low yield and fiber quality. Long staple cotton Preferred in textile industry due to ease of spinning and weaving. Sucrose Synthase (SuS) catalyzes the reversible cleavage of sucrose into UDP into fructose and UDP-glucose. The current study deals with Agrobacterium mediated transformation of SuS gene into a local cotton variety for improvement of fiber quality. Removal of 2815bp fragment by restriction digestion with specific restriction enzymes ensures successful ligation of fiber gene into pCAMBIA 1301. Successful transformation was confirmed through PCR and GUS assay. Transgenic plants showed increased vegetative growth, high SuS activity in fiber and increased cellulose contents as compared to control plants. Real time PCR analysis showed increased SuS expression at vegetative and fiber stage. Scanning Electron Microscopic analysis of mature fiber showed that fiber of transgenic plants is smooth and more twisted. Incorporation of genes related to cotton fiber length and quality can provide new avenues for fiber improvement. The utilization of this technology would provide an efficient import substitution and sustained production of long staple fiber in Pakistan to fulfill the industrial requirements.

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Lignin Down-regulation of Eucalyptus by RNAi

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Ethanol production today is mostly from edible crops by fermentation of sugars from sugarcane or conversion of starch into sugars from corn. Ethanol production by using edible crops has been referred as "first generation" biofuels which is correlated with "food versus fuel". This conflict has led a surge in research interest for finding alternative biomass resources to produce biofuels and is referred as "next generation biofuels". Next generation biofuel production is mainly based on cellulose. Selection of source of lignocellulosic biomass for commercial fuel production is crucial. Keeping in view the current requirements of the next generation biofuels, the present study is aimed to down-regulate lignin contents using RNAi. Eucalyptus species are famous for their fast growth, high yield, short rotation period and its capability to adapt a wide range of environments. Cinnamoyl-CoA reductase is one of the key enzymes involved in lignin biosynthesis pathway. Therefore, RNAi construct of CCR was developed and transformed into Eucalyptus by Agrobacterium mediated method of transformation. Initially putative transgenic plants were screened using Basta selection marker. Confirmation of transgene was done using genomic DNA PCR and Reverse transcriptase PCR. Lignin estimation of transgenic and untransformed control was done following NREL method of lignin estimation. Highest Lignin content down regulation was observed in transgenic event number 13 and 21 which was 17.98% and 17.26% respectively. This lignin content was 38.5% to 40.95% less than the lignin content of non-transgenic tissue cultured plants and data was generated from 18 months old plants.

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Affinity Of The Chitin Of Insects With Epicuticular Wax Compounds Of Cotton

Muhammad Azmat Ullah Khan[1], Ahmad Ali Shahid[2], Abdul Qayyum Rao[3], Muhammad Azam Ali*[2], Muniba Abid[3], Amina Arif[1], Sabiha Rashid[1] and Tayyab Husnain[2]

Plant epicuticular waxes plays important role in protecting the plants from both abiotic and biotic stress. Being uppermost layer on the plants, these waxes are the first thing that may facilitate the insects or may cause hindrances for attachment by interacting with insect's uppermost layer i.e., chitin. This study is focused on the possible interaction of wax compounds of selected species of Gossypium with chitin of the insects. GC-MS analysis of epicuticular waxes of G.hirsutum, G. arboreum, G. harknessii and Gossypium arboreum wax deficient mutant (GaWM3) depicted that some of the compounds are common while few compounds are specie specific. P-xylenolpthalein and Trichloroacetic acid, hexadecylester were only present in G. hirsutum and have great affinity with chitin. The compund 2-cyclopentene-1-ol, 1-phenyl- which is present in G. arboreum and G. hirsutum also shows affinity with chitin. Phenol, 2,5-bis [1,1- dimethyl] is present is G. arboreum, GaWM3 and G. hirsutum. The compunds 2-piperidinone, n-|4-bromo-n-butyl| and Lanceol, cis- are present only in G. arboreum and G. harknessii respectively also show affinity with chitin whereas rest of the detected compounds act neutral, they neither attract nor repel the insect for attachment. From the above study it was conclude that plants that contain more epicuticular wax compounds which can have affinity with chitin are more susceptible to insects whereas the plants which have least such compounds are somewhat resistant to insects.

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Antihyperglycemic and antihyperlipidemic potential of *Myrica esculenta*

Shahzada Khurram Iftikhar*[1], Shoaib Ali[1] and Muhammad Farooq[2]

Medicinal plants and their derivatives are in use from very early times to control diabetes mellitus. Complications and exact effectiveness of these drugs are still not completely known. Many works were carried out on practicing medicinal plants on rats but use of such plants can be better understood by applying them on humans. Present investigation is carried out on diabetic Type-II patients. A questioner was designed in which age, sex, personal history, family history and prescribed medication was recorded. Members with age above 35 and fasting glucose level 135mg/dl were included in the study. A consent letter and contact numbers were also taken from patients and were in regular contact to avoid any serious condition. Patients with hypoglycemic drugs were allowed keeping on their therapy. A total of 12 patients were selected for enquiry from both genders. Present investigation reveal that Myrica esculenta reduce 22% glucose level, 7.6% triglycerides, 12.9% cholesterol, and 21.5% LDL cholesterol level in diabetic patients. This investigation can be helpful in controlling diabetes mellitus in cheap price and without any side effect.

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Molecular characterization of important fungal pathogens infecting mungbean crop from Pakistan

Shamila Hussain*, Nazia Nahid* and Tayyaba Huma*

Mungbean (Vigna radiata) is an easily digestible pulse crop that is rich in dietary protein. Average yield of mungbean in Pakistan is much lower than other countries due to the cultivation on marginal lands, low inputs and diseases attack. Fungal diseases cause grain yield loss of mungbean crop up to 60% in Pakistan. Among fungal pathogens, Alternaria and Curvularia cause severe leaf damage and yield loss. Present study was conducted for the characterization of these fungal pathogens on morphological basis and at molecular level. The mungbean field areas of Faisalabad were surveyed during the cropping season in year 2016. The leaf samples with leaf spot disease symptoms were collected. After surface sterilization with 15% sodium hypochlorite the infected leaf patches were grown on potato dextrose agar (PDA) media at 28℃for five to seven days. Sporulation pattern, morphology of colony and characteristics of conidiophores and conidia were studied under light microscope. One of the isolate SH1-MB was identified as a member of Alternaria species and the other isolate SH2-MB was belonged to Curvularia species on the basis of morphological and culture characteristics. For molecular characterization, the internal transcribed spacer (ITS) region for both isolates was amplified using ITS1 and ITS4 primers. The DNA sequencing of amplified fragments was done from macrogen USA. BLAST analysis of the DNA sequences was performed which revealed that the ITS region from SH1-MB strain is 99% identical to A. alternata and A. tenuissima (Alternaria section Alternaria) whereas the isolate SH2-MB is 100% identical to Culvularia species. Based on these findings it was concluded that the pathogens involved in leaf spots symptoms are the species of Alternaria and Curvularia. This is the first report about the infection and molecular characterization of Alternaria and Curvularia species on the mungbean plants.

ABSTRACT INFO

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Transformation of Chitinase-II gene in local maize (*Zea mays* L.) inbred lines

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Agrobacterium mediated transformation was performed to introduce fungal resistant gene into the immature embryos of corn. For transformation, barley chitinase II gene of 690bp was transformed into the plant by using the pCAMBIA 1301 vector under control of ubiquitin promoter. Embryos were grown over the induction and then regeneration media. Presence of gene was confirmed in the regenerated transgenic maize plants through PCR by amplification. A sharp band of 690bp was observed in maize transgenic plants. Further, for the confirmation of stable gene integration in positive transgenic plants Dot blot analysis was performed, which showed the presence of band on the membrane. Maize line CEMB-44 showed transformation efficiency up to 1.7%. Histochemical GUS assay and fungal inhibition assay were also carried out. Transgenic crude protein extracted from CEMB-44 transgenic plants showed 49% of fungal inhibition percentage. Transgenic maize plant was selected for the evaluation of whole plant resistance to Fusarium verticilliodes along with non-transgenic plant, through in-vitro anti-fungal bioassay. After 2 weeks, control plant turned brown and dried but CEMB-44 transgenic plant remains green. From the results, it was concluded that barley chitinase II gene when transformed in maize, developed resistance against ear rot disease of corn.

ABSTRACT INFO

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Enhancing abiotic stress tolerance in local varieties of cotton by manipulating the stress resistance genes

Muhammd Nadeem Hafeez, Sameera Hassan*, Bilal Sarwar*, Fatima Batool*, Quban Ali*, Bushra Rashid*, Tayyab Husnain* and Mohsin Ahmad Khan*

Local variety of cotton G. *arboreum* is rich source of biotic and abiotic stress tolerant genes, 80% of its genes are uncharacterized. Current research is first attempt towards the characterization of full length Gossypium universal stress protein-2 (GusP2) gene by point mutated at three different positions and scrutinizing the mutated (M1Usp2-Mu-352-354-Lysine-60-Proline, M2Usp2-Mu-214-216-Aspartic Acid-26-Serine, M3Usp2-Mu-145-147-Lysine-3- Proline) and wild type (wUsp2) genes in Pichia pastoris and E.coli under various abiotic stress (salt-800mM, heat-37-480C, osmotic-8%PEG and cold-40C) conditions before final validating best mutated GUSP-2 gene in G. *hirsutum* under drought stress conditions as compared to wild type and other mutated genes. It was observed under scrutinizing experiments in Pichia pastoris and E.coli mutant-1 of GusP2 (M1Usp2) enhanced the tolerance of recombinant cells under salt and osmotic stress conditions but no resistance was found towards cold and heat stress treatments. The recombinant cells transformed with M1Usp2 genes showed growth up to dilution 10-5 in spot assay as compared with wild type (wUsp2) and other mutated genes (M2Usp2, M3Usp2). The transcript profiling of M1Usp2 gene in Pichia pastoris exhibited noteworthy expression under salt (7.2folds) and osmotic (9.8folds) stress treatments. However M2Usp2 gene was found less significant to imparting tolerance towards osmotic and salt stress conditions in both E.coli and Pichia pastoris. M1Usp2 gene was also significantly active in enhancing the drought tolerance capacity of transgenic CIM-496-G.hirsutum plants as compared to control and plants transformed with wild type wUsp2 gene. It was concluded that the activity of GusP2 gene can be enhanced in M1Usp2 mutant form by enhancing its ATP-binding capacity and its activity could be wiped out in M2Usp2 with zero ATP-binding capacity. In silico analysis predicted that M1Usp2 gene might be directly involved in stress tolerance or work as signaling pathway to activate stress tolerant mechanism

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Genetic Diversity of Soybean Cultivars with reference to Meloidogyne incognita

Musarrat Ramzan Arain*

Investigations in green house are useful whenever data required for further screening of nematode populations or may be compared different nematode populations and or plant species / cultivars. These studies were conducted to evaluate the resistance of M.incognita at different level of inoculums in the soybean (Glycine Max L) in green house conditions. Moderately resistance and susceptible cultivars may exhibited the same or vary close tolerant limit (T) but show minimum yield, in this way use of several inoculums levels would differentiate various nematode population or tested plant on the basis of the growth reduction occurring at initial population (Pi) larger than tolerance (T). Out of fifteen varieties of soybean only six varieties were found resistant in green house trial viz., AGS-08 (V9), AGS-09 (V5), FS-85 (V6), NARC-3 (V11), 95086 (V8), NARC-5 (V13), and Rawall-I (V14) and rest showed susceptibility to root-knot nematodes. Molecular marker analysis is useful for identification of genomic segments contributing to the genetic variance of a trait and selection of superior genotypes. Marker analysis provides accurate genotypic information, and gives precision which lacks in phenotypic measurements due to environmental interaction and experimental error. The present investigation has been carried out to evaluate the usefulness of molecular markers RAPD for revealing the level of genetic diversity among the Mi resistant and susceptible cultivars of soybean collected from Pakistan and United State soybean germplasm collection USDA. Indigenous soybean cultivars exhibited a low level of variation at DNA level by RAPD that has been the most successful test in identifying genetic variation within the Mi resistant soybean species / cultivars.

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Diverse genetic reflex by the *Agave sisalana* transcriptome in response to drought Stress provides insight to the tolerance mechanisms

Muhammad Bilal Sarwar, Zarnab Ahmad, Bushra Rashid*, Sameera Hassan, Nadeem Hafiz, Per L Gregersen, Tayyab Husnain

Agave plants are adapted to survive in the extreme harsh desert environment with typical drought and cold conditions. They employ different strategies to overcome these stress factors like crassulacean acid metabolism, wax deposition, typical leaf morphology, etc. To understand the role of changes in gene expression levels as a part of these strategies, we sequenced the transcriptome of well-irrigated and drought-treated samples of Agave sisalana in replicates and obtained 280 million high-quality paired-end reads. Different de novo assembler like Trinity, MIRA V.4 and trans-AbYss with different kmer length (25-64) were used to construct assemblies. After qualitative and quantitative analysis, we chose the assembly developed by Trinity for further downstream analysis. We obtained total 93644 contigs with an average length of 780 bp. Before differential gene expression analysis, we cleaned our de novo assembly from the occurrence of other genes than those of plant origin based on homology searches against the nr database from the NCBI. After cleaning from alien contigs, functional annotation was obtained by aligning all Unigene with public protein databases including nr, SwissProt, KEGG, and COG. Differentially expressed genes (DEGs) were investigated using the RSEM method. Further analysis of these DEGs gene is underway. This study will help us in the identification of the genes and transcription factors from Agave sisalana species against drought stress.

ABSTRACT INFO

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TRANSGENIC EXPRESSION OF GLYPHOSATE TOLERANT (CEMB-gtgene) AND CANE BORER RESISTANT GENES (CEMB-Cry1Ac, CEMB-Cry2A) IN SUGARCANE (*Saccharum officinarum* L.)

Zahida Qamar*, Idrees Ahmad Nasir and Tayyab Husnanin

Sugarcane (*Saccharum officinarum* L.) is an economically important cash crop. Quality yield is threatened by the damages of cane borers (Chilo Infuscatellus) and weeds. Two problems were addressed through the expression of modified two cane borer resistant genes CEMB-Cry1Ac, CEMB-Cry2A and glyphosate tolerant CEMB-GTGene. For higher expression, modified synthetic genes were designed according to the sugarcane genome by using the Codon optimization tool of Integrated DNA Technologies (IDT). One Variety was screened for further generation's level field study from SPF-213, SPF-234, HSF-240, and CPF-246, through tissue culturing response, glyphosate spray assays and transformation efficiency. Double selection (Kanamycine (50mg/L) + glyphosate (50mM) gave 34% of SPF-213, 40% of SPF-234, 29% of HSF-240 and 81% of CPF-246 resistant calli. These transgenic shoots were confirmed through GUS assay and PCR analysis by using glyphosate gene-specific primers. The transgenic plants were treated with three doses, 900mL/acre, 1100mL/acre and 1200mL/acre of glyphosate sprays. The transformation efficiency 1.1% for SPF-213, 1.3 for SPF-234, 1% for HSF-240, and 1.5% for CPF-246 was observed. In V0, V1 and V2 generations, confirmation for stable integration of the transgenes was carried out by Sothern Blot, Dipstick assay and Enzyme-Linked Immunosorbent Assay (ELISA), Biotoxicity assays and glyphosate sprays. It was concluded that with the expressions of CEMB-Cry1Ac, CEMB-Cry2A, and CEMB-GTGene, genes sugarcane variety CPF-246 efficiently resist against the cane borers (70-100% mortality) and was highly tolerant for glyphosate spray assay (1200mL/acre). These advanced lines can be very helpful for achieving higher and sustainable yields free of insect pests and weeds damages.

ABSTRACT INFO

Session: Molecular Forensics

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FORENSIC EVALUATION FOR DETECTION AND QUANTIFICATION OF PESTICIDES RESIDUES FROM AGRICULTURE SAMPLES IN LOCAL MARKETS OF LAHORE

Sara Mehboob, Muhammad Farhan Khan, Muhammad Taimoor Chaudhary, Mohammad Sarwar, Ahmed M. Khalid

Pesticides kill insects and save farms from their unwanted action to increase the food production. The leftover of pesticides are called pesticides residues. Pesticides have variable classes depending upon their mechanism of pest control. Fruits and vegetables play a major role in the economy of Pakistan. Pesticides leave their residue on fruits and vegetables, hence rendering them unhealthy to consume. In this research, different fruits and vegetables were collected from markets of Lahore and rinsed with dichloromethane and ethyl acetate to collect pesticide residues present on their surfaces. GC/MS scan mode was run for identification of pesticides. GC/MS SIM mode was run for all pesticides and one qualitative and two quantitative ions were found. All of the samples were contaminated with one or more pesticides. Chlorpyrifos, pryipoxyfen, lambda cyhalothrin and Endosulfan were found in maximum number of samples and for all samples, the residues were above European Commission provided Maximum residual level. This alarming trend requires strict control over pesticides use.

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