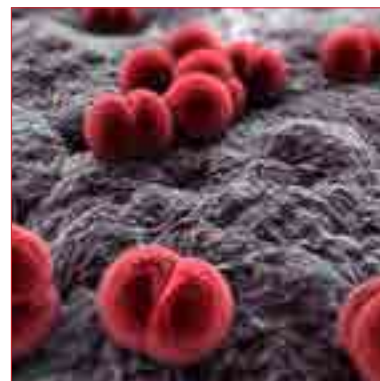
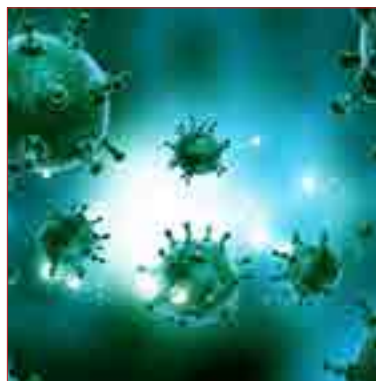


Poster

***Biotechnology 2017***



**ANNUAL BIOTECHNOLOGY CONGRESS**

August 17-18, 2017 | Holiday Inn Toronto International Airport  
Toronto, Canada

# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Beta defensin 3 enhances ovarian granulosa cell proliferation via MAPK pathway

Canying Liu<sup>1,2</sup>, Bo Pan<sup>2</sup>, Bingyun Wang<sup>1</sup> and Julang Li<sup>2</sup>

<sup>1</sup>Foshan University, China

<sup>2</sup>University of Guelph, Canada


Antimicrobial peptides (AMPs) are regarded as host defense peptides which processes bactericidal, and they have also been reported to have immunomodulatory function in many tissues. However, their role in the mammalian ovary is unknown. We have previously found that beta defensin 2 (BD2) and beta defensin 3 (BD3) are expressed in granulosa cells and cumulus cells during porcine ovarian follicular development and cumulus-oocyte-complex maturation. We hypothesized that these antimicrobial peptides are involved in the regulation of follicular development in ovaries. Granulosa cells were isolated from small (1-3 mm in diameter) and large (4-8 mm in diameter) porcine follicles and cultured in the absence and presence of 1, 10, and 50 µg/ml of BD2, and BD3. After 24 hours of treatment, cell numbers were counted using an automated cell counter. It was found that while BD2 appears to have no effect, BD3 stimulated granulosa cell proliferation in a dose dependent manner ( $p < 0.05$ ). This effect is also dependent on the stage of follicular development, as it is effective on granulosa cell from small but not large follicles. In addition, transwell cell migration assay revealed that in the presence of BD3 (10 µg/ml), a

2.5 fold increase in cell migration was achieved. To further study the potential pathway involved in BD3 induced cell proliferation; western blots were performed to determine the ratio of phosphorylated- and non-phosphorylated-ERK1/2. It was found that BD3 significantly increased the phosphorylated-ERK1/2 ratio. Moreover, U0126, the specific ERK1/2 phosphorylation inhibitor, suppressed BD3 induced ERK1/2 phosphorylation and proliferation, suggesting that BD3 may stimulate granulosa cell proliferation via activating the MAPK pathway. Our data suggests that antimicrobial peptides may play a physiological role, in addition to being the traditionally recognized immune-defense mechanism, in regulating follicular development in the mammalian ovary.

### Speaker Biography

Liu Canying has completed her PhD at the age of 28 years from Huazhong Agricultural University College of Veterinary Medicine. She has just graduated and been the lecturer of Foshan University Department of Veterinary Medicine for about one year. She has published 4 papers in famous journals. She mainly performed research projects about the comparative genomic and pathogenicity analyses of bacteria, immunogenicity analyse of outer membrane proteins of bacteria.

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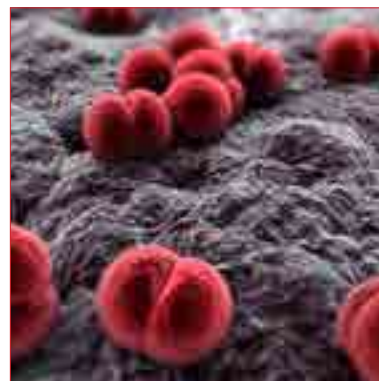
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# e-Posters

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## *Biotechnology 2017*



# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Holiday Inn Toronto International Airport  
Toronto, Canada

# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Prevalence of Epstein–Barr virus genotypes in Pakistani lymphoma patients

Sadia Salahuddin<sup>1, 2, 3</sup>

<sup>1</sup>Cornell University, USA

<sup>2</sup>The University of North Carolina, USA

<sup>3</sup>Gomal University, Pakistan


The Epstein-Barr virus (EBV) is a herpes virus infecting more than 90% of the human population. The tropism of EBV for B lymphocytes is evidenced in its association with many lymphoproliferative disorders. Different types of EBV (EBV-1 and EBV-2), classified on the basis of EBNA-2 genotyping, have been reported in benign and malignant pathologies, but there is almost no information about their frequency in the Pakistani population. The aim of this study was to determine the frequency and distribution of EBNA-2-based EBV genotypes in lymphoma patients. Genomic DNA was extracted from formalin-fixed paraffin embedded (FFPE) tissue samples obtained from 73 EBV-DNA-positive lymphoma patients. The  $\beta$ -globin gene was amplified to assess the presence and quality of cellular DNA from all samples. EBER-1 DNA was detected by PCR to confirm EBV presence in tissue samples. EBNA-1 mRNA relative quantification by quantitative PCR substantiated EBNA-1 mRNA

overexpression in 43.8% of EBV-positive cases in comparison to an EBV-positive control cell line. EBNA-2 genotyping was done by nested polymerase chain reaction (PCR). Among the samples, EBV-1 was present in 90.7% and EBV-2 in 9.3%. These results show that EBV-1 is the most prevalent type in the lymphoma population of Pakistan, similar to reports from other countries. This definition of EBV epidemiology in Pakistani lymphoma patients represents an important first step in using EBV for prognosis and monitoring treatment response in patients.

### Speaker Biography

Sadia Salahuddin is PhD scholar at Gomal University. She worked at Cornell University and University of North Carolina as research scholar for four years. She has authored number of good quality research articles in reputed journals and has been serving as an editorial board member of reputed journal.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Renovation of the injured myocardial tissue with new conductive, biodegradable, and non-cytotoxic polymer matrix coated with fibrin glue nanocomposite scaffold

Sharareh Ghaziof<sup>1</sup> and Mehdi Mehdikhani-Nahrkhalaji<sup>2</sup>

<sup>1</sup>Islamic Azad University, Iran

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**A**cute myocardial infarction (AMI) occurs when a coronary artery is clogged, in 80% of the cases, by coronary atherosclerosis with superimposed luminal thrombus. This occlusion leaves the downstream zone of the heart without blood supply. As a result, the papillary muscles are separated, what leads to regurgitation, contributing to the overload of the heart. Cardiac muscle engineering aims at providing functional myocardium to repair diseased hearts and model cardiac development, physiology, and disease *in vitro*. The objective of the present *in vitro* study was to prepare, characterize and assess polycaprolactone (PCL)/fibrin glue (FG)/multi wall carbon nanotube (MWCNTs) nanocomposite scaffolds, to guide regeneration of myocardial tissue. For this purpose, two different weight ratio of multi wall carbon nanotubes (1 and 0.5 % wt) were added to the pure PCL polymeric scaffold by solvent casting process. The nanocomposite scaffolds were coated by fibrin glue and the solvent was removed from the structure by freeze drying technique. Characterization technique such as Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), FT Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD) were performed. Tensile tests were carried out for evaluating the mechanical properties. To evaluate the cytotoxicity of scaffolds, MTT assay were performed with mouse myoblast cells in 1, 4 and 7 days. Biodegradability, electrical conductivity as well as contact angle and wettability of the nanocomposite scaffolds were investigated. Carbon nanotubes have crystalline structure, electrical conductivity,

and particle size was in the range of 20-30 nanometer. Both coated and uncoated nanocomposite scaffolds showed appropriate cell response during the period of specified times. Meanwhile, the adhesion of the cells was more for coated nanocomposite scaffolds. Addition of MWCNTs to the pure PCL polymeric scaffolds significantly raised the electrical conductivity. MWCNTs have a good adhesion with fibrin glue in coated samples. In the presence of carbon nanotubes, the elastic modulus of the nanocomposite scaffolds compare to the pure PCL polymeric scaffolds were increased. In vitro degradation assessment exhibited that samples had significant weight loss after two months and the degradation of the samples were increased not only by adding MWCNTs but also by coating the samples with fibrin glue. In the presence of fibrin glue, nanocomposite scaffolds became hydrophilic and contact angle was decreased. It was concluded that bioactive, degradable and electrical conductive nanocomposite scaffolds made of polycaprolactone/fibrin glue/multi wall carbon nanotubes could be used as an appropriate construct for reconstruction and restoration of damaged myocardial tissue..

### Speaker Biography

Sharareh Ghaziof has completed her Master's degree in Biomedical-Tissue Engineering from Islamic Azad University, Najafabad Branch, Najafabad, Iran. She has developed her passion for academic research and experiences in tissue engineering, drug delivery and related topics at University of Isfahan and Isfahan University of Medical Science (Central laboratory, School of Medicine).

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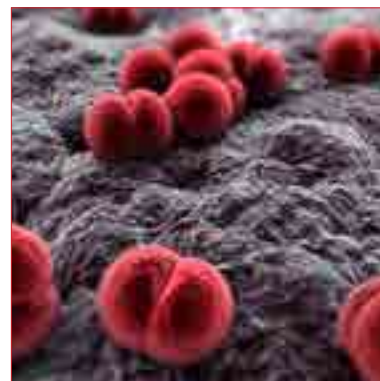
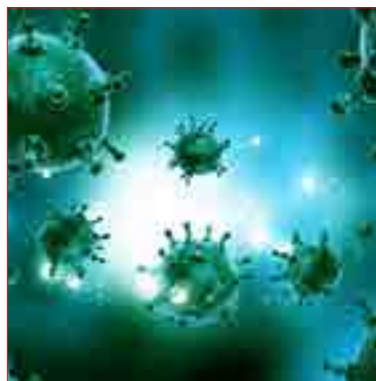
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# Accepted Abstracts

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## *Biotechnology 2017*



# ANNUAL BIOTECHNOLOGY CONGRESS

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Malignant hyperthermia: the motor unit potential (MUP) predicts susceptibility to developing the MH syndrome

Charles H Williams

The Williams Research Laboratory, USA

Mating MH positive pigs to MH positive pigs produces an F1 generation that is highly stress susceptible. Recording the MUP shows MH + pigs have a higher U voltage than control pigs. Older MH+ pigs have an even higher U voltage than control pigs. The duration of the voltage spike is also increased in MH+ pigs versus control pigs and older MH+ pigs have even a longer duration of the voltage spike. We can assume that by concentrating the MH genetic defect in the F1 generation that the population of defective sodium channels in the acetylcholine receptor was present at a high concentration. Since the acetylcholine receptors are spatially located under the foot piece of the myoneural junction which makes them a bank of receptors that are readily

accessible when acetylcholine is released by the nerve, and the action of acetylcholine is very rapid. Therefore, the electromyographic data reflects the genetically defective sodium channels as the major functional component when we recorded the data. The sodium channels can be likened to a low voltage switch in a telephone circuit that is used to route telephone calls. We would suggest that the sodium channel at the acetyl choline receptor has been adapted to produce heat as well as muscle contraction and that the ability to produce copious amounts of heat is the biological mechanism that differentiates warm blooded animals from cold blooded animals.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Rice cultivation and greenhouse gas emissions: a review and conceptual framework with reference to Ghana

George Yaw Obeng  
Arizona State University, USA

Rice is an essential crop in Ghana. Several aspects of rice have been studied to increase its production; however, the environmental aspects including impact on climate change, have not been studied well. There is therefore a gap in knowledge, and hence the need for continuous research. By accessing academic portals, such as Springer Open, InTech Open, Elsevier, and the Kwame Nkrumah University of Science and Technology's offline campus library, 61 academic publications including peer reviewed journals, books, working papers, reports, etc., were critically reviewed. It was found that there is a lack of data on how paddy rice production systems affect greenhouse gas (GHG) emissions, particularly emissions estimation, geographical

location, and crops. Regarding GHG emission estimation, the review identified the use of emission factors calibrated using temperate conditions which do not suit tropical conditions. In terms of location, most research on rice GHG emissions have been carried out in Asia with little input from Africa. In regard to crops, there is paucity of in-situ emissions data from paddy fields in Ghana. Drawing on the review, a conceptual framework is developed using Ghana as reference point to guide the discussion on fertilizer application, water management rice cultivars, and soil for future development of adaptation strategies for rice emission reduction and increase in yield.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Development of enzymatic membrane bioreactors for removal of pharmaceuticals from wastewater

Faisal I Hai

Univeristy of Wollongong, Australia

Pharmaceuticals are ubiquitously detected in wastewater and wastewater-impacted waterbodies because they are resistant to bacterial degradation and thus pass through conventional wastewater treatment systems. This raises significant concern about their potential harmful impact on aquatic organisms and even human. Fungal laccase (EC 1.10.3.2) can degrade pharmaceuticals but its application is limited because of the concern of washout of laccase along with treated effluent from a continuous flow bioreactor. We previously developed an enzymatic bioreactor coupled with an ultrafiltration membrane, which prevented enzyme washout, thereby allowing continuous enzymatic degradation of pharmaceuticals. We noted that some resistant compounds such as naproxen and salicylic acid were retained by an enzyme gel layer formed on the membrane surface, subsequently resulting in their

enhanced biodegradation. Based on this observation, we postulate that integration of high retention membranes with an enzymatic bioreactor can facilitate biodegradation of recalcitrant compounds by retaining both enzyme and the pharmaceuticals. This study explores a novel membrane distillation-enzymatic bioreactor system for the removal of four pharmaceuticals namely, diclofenac, naproxen, salicylic acid and ibuprofen as well as two ingredients of personal care products namely, oxybenzone and salicylic acid using laccase purified from genetically modified *A. oryzae*. The results confirmed almost complete retention (>95%) of the compounds by the bioreactor. Of particular interest was the fact that the complete retention improved the enzymatic degradation of compounds that have been reported to be poorly removed in other enzymatic bioreactors.

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# ANNUAL BIOTECHNOLOGY CONGRESS

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## Multi-purpose and non-invasive methodology and the main goal for biotissues speedy regeneration

Elena V Orlova

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The main goal and the core methodology of the present research is the speedy and non-invasion recovery of wide variety of biotissues materials: from cell cultures upto organism level. There are two parts of our technology: extra- and intra- influence on a damaged biomaterial. Here, extra-influence means weak low frequency magnetic field with special characteristics for the exact biomaterial and intra means artificial interstitial matrix gel substance. It was shown that the above technology could be suitable for stem cells speedy growth and regulation of their differentiation; for acceleration of the healing of chronic persistent and/

or septic wounds, healing of burns, wound caused by metabolic disorders (diabetes and foot problems), *in vitro* embryos cultivation, prevention (on genome level) of stem cells transformation into cancer cells, speedy cultivation and restoration for freezed cell cultures. So, this technology could be applied to many branches of biology and medicine, such as cell therapy, transplantation, regenerative medicine, growing of artificial organs, 3D-bioprinting; in surgery and traumatology, but also in the recovery of rare and endangered species as a environmental sustainability tool.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Plant made pharmaceuticals for developing countries

**Kathleen Hefferon**

University of Toronto, Canada

**P**lant made biologics have elicited much attention over recent years for their potential to assist those in developing countries who have poor access to modern medicine. Vaccines and other biopharmaceuticals derived from plants are inexpensive, lack refrigeration requirements and can be produced en masse in a relatively short period of time. Pharmaceuticals developed in this fashion could be utilized for functions ranging from defense against infectious diseases that have pandemic potential, such as influenza or Ebola virus, to combating orphan diseases which are poorly

funded yet remain paramount to global health in their respective endemic regions. Biopharmaceuticals have been generated via many plant production platforms, including stable expression in transgenic plants, suspension cell cultures and hairy roots, as well as transiently using plant virus expression vector technologies. The presentation will provide an overview of plant-derived pharmaceuticals and will conclude with a projection of the impact they could have for developing countries.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## How molecular biology can help in finding the origin of genetic defects in different populations

Mehrez M Jadaon

Kuwait University, Kuwait

Molecular biology has become an integral figure in the modern medicine, making DNA analyses as essential tools in the diagnosis, prognosis and management of diseases. Factor V Leiden (FVL) is the name of the most common genetic mutation which is associated with venous thrombosis, a disease that has high morbidity and mortality rates. Earlier studies on FVL found it in Europeans only, bringing speculations that FVL had occurred as a single event in the far past, in a single European ancestor who fixed the mutation in the current European carriers. However, our later research in Kuwait proved the presence of FVL in different non-European populations living in Kuwait. Does this mean these were originally from Europe? In this research, we showed how molecular techniques were used to explore the origin of FVL in different populations living in Kuwait. Was it the same European ancestor, or a separate one? A total of 512 healthy individuals were recruited from different populations (non-European) living in Kuwait: 360 Arabs (Kuwaitis, Lebanese, Syrians, Jordanians, Saudis, Iraqis, Palestinians, and Yemenites), 102 Armenians and 50 Afghans. A blood sample was collected from each case which

was used for DNA extraction. Real-time PCR was performed on the DNA samples to test for the presence of FVL. In the positive cases for FVL, real-time PCR was performed to explore 9 single nucleotide polymorphisms (SNPs) in the Factor V gene; these SNPs were previously reported to be associated with FVL in European carriers of the mutation (in linkage disequilibrium with FVL). The same was done on a randomly selected number of the negative cases (equals to the number of the positive cases). 99 of the 512 cases were found positive for the FVL mutation. When the 9 NPs were analyzed, all our positive cases had the same 9 alleles that were present in Europeans. However, this was not true in the negative cases. The results indicate that our positive cases in Kuwait had most probably descended from the same proposed European ancestor who had the FVL mutation event. Further studies are planned to perform additional molecular tests and combine our results with the available epidemiological data and anthropological knowledge to possibly determine how this mutation had reached Kuwait.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Developing human and human-like antibodies for biodefense

Michael Hust

Technische Universität Braunschweig, Germany

Antibody phage display is a key *in vitro* technology to generate human antibodies. In this presentation, our pipeline for the development of human and human-like antibodies for diagnostics and therapy will be outlined. The development of human and macaque antibodies against category A and B (according to CDC) classified viruses and toxins using phage display will be presented. Detailed

examples will be given for diagnostic and neutralization of *Venezuelan equine encephalitis virus* (VEEV), *Western equine encephalitis virus* (WEEV), Marburg virus and botulinum toxins (EU FP7 Antibot ABE consortium). In addition, the germline humanization of the anti-botulinum toxin antibodies will be outlined.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Adipose-derived cells for clinical bone regeneration: a concept in motion

Arnaud Scherberich  
University of Basel, Switzerland

This lecture will review how biomaterials and components of the extracellular matrix, i.e. structural proteins and growth factors, affect the osteogenic potential of human adipose-derived mesenchymal stromal cells (ASC). Examples of bone formation by various human adipose derived cells-based engineered matrix/tissue, via either intramembranous or endochondral ossification will be presented. The lecture will then present the development of an advanced therapy medicinal product (ATMP) based on an intraoperative use of the stromal vascular fraction (SVF) of human adipose,

containing mesenchymal and endothelial cells, to support bone repair with tissue harvest, cell isolation, seeding onto scaffolding material and implantation within 3-4 hours. A translation of this concept into a first-in-man clinical trial, demonstrating safety, feasibility and providing proof-of-principle of the biological functionality (i.e., bone formation) of the implanted graft will be presented. Another clinical case based on the use of such ATMP for mandibular bone regeneration will be shown.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## On-site removal of antibiotics and antibiotic resistant genes from leachate by aged refuse bioreactor: effects of microbial community and operational parameters

Bing Xie and Yinglong Su  
East China Normal University, China

The abuse of antibiotics has raised the prevalence of antibiotic resistance, and the high frequency of antibiotic resistance will be a serious global health concern. Landfill is the primary treatment for municipal solid waste, and the generated leachate will be the important hotspot of the antibiotics and antibiotic resistant genes (ARGs). Until now, no effective on-site treatment has been put forward for preventing ARGs dissemination during leachate treatment. Herein, the aged refuse bioreactor was employed to remove antibiotics and ARGs from leachate, and the great removal performance was observed. For the detected antibiotics, the total removal efficiency was about 76.75%, and sulfanilamide and macrolide were removed with high efficiencies (>80%). Among the target ARGs, tetracycline and macrolide resistance genes (*tetM*, *tetQ* and *ermB*)

were eliminated with 1.2-2.0 orders of magnitude. The occurrences of ARGs did not correlate with physicochemical parameters, but closely linked to the variations of the bacterial community structure. Redundancy analysis (RDA) indicated the significant correlations between four genera and the distribution of ARGs, which implied that these key genera (including potential pathogens) drove the ARGs removal. Furthermore, the hydraulic loading test confirmed that the aged refuse bioreactor was capable of achieving high removal efficiencies even under shock loading and for the higher loading it was negative for the proliferations of potential ARGs hosts. This study suggested that aged refuse bioreactor could be a promising way for antibiotics and ARGs on-site removal from leachate.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Hedgehog signaling scenario in the hematopoietic microenvironment of chronic agricultural pesticide induced aplastic bone marrow

Sujata Law

Calcutta School of Tropical Medicine, India

Overactivation of hedgehog signaling has been found to be associated with a wide variety of hematological and nonhematological malignancies including cancer stem cells. However, involvement of the hedgehog signaling system in bone marrow hematopoietic microenvironment during the progression of the bone marrow aplasia is absolutely unknown. In the present work, we have developed an agricultural pesticide formulation (fungicide, organophosphate and pyrethroid) induced bone marrow aplasia mouse model to recapitulate the human aplastic anemia like condition in the laboratory to study the aplastic hematopoietic microenvironment in the light of HH-GLI signaling pathway. Our study has unfolded the fact that chronic pesticide exposure caused downregulation of intrasignaling feedback of PATCH1 and GLI1 by inhibiting the SMO internalization and upregulating downstream negative regulators SU(FU), PKC- $\delta$  and  $\beta$ TrCP. Upregulation of negative regulators not only hampers the execution of the hedgehog signaling but also cripples the autocrine-paracrine crosstalk

in between bone marrow primitive compartment and stromal compartment. Simultaneously, individual pesticide versus hedgehog signaling study revealed that hexaconazole disrupted hematopoietic hedgehog signaling activation by inhibiting SMO and facilitating PKC- $\delta$  expression. Contrarily, chloropyrifos increased the cytoplasmic sequestration and degradation of GLI1 by upregulating SU(FU) and  $\beta$ TrCP sequentially. Whereas, cypermethrin mediated antagonization of the hedgehog signaling was circumvented by noncanonical activation of GLI1. However, such marrow degenerative condition can be compensated by the recombinant sonic hedgehog. We can conclude that pesticide exposure induced bone marrow aplasia is the direct manifestation of downregulated hedgehog signaling in the bone marrow microenvironment and application of recombinant sonic hedgehog could be a way to improve the overall scenario.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Development of glyphosate-tolerant transgenic potato plants harboring the G2-aroA gene

Arfan Ali<sup>1,2</sup>

<sup>1</sup>Fb Genetics Limited, Lahore, Pakistan

<sup>2</sup>University of the Punjab, Pakistan

Glyphosate weed control is a very effective strategy to minimize cost and improve economic outcomes of world and Pakistan agriculture production. Development of glyphosate-resistant potato hold great promise. A new G2-aroA gene from *Pseudomonas fluorescens* which encodes 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) was transformed by using an *Agrobacterium*-mediated transformation into potato cultivar AGB-Red. Transgenic potato plants were generated via node tissue culture method, using kanamycin selection. Ten regenerated potato plants were obtained and allowed to grow normally in pots under

normal conditions. The Polymerase Chain Reaction (PCR), Southern Blotting and Western Blotting analysis confirmed that the target gene was integrated and expressed effectively into potato chromosomes at the very potential level. The glyphosate tolerance assay showed that transgenic potato had a high resistance level to glyphosate. Furthermore, potato plants treated with 50.0 mmol/L of glyphosate could grow slowly and can develop tubers. It was concluded that transgenic potato may be used for cotton breeding research of glyphosate-tolerant potato.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Morphogenesis and histology of cultures of *Iris ensata* Thunb. generative organs

L Tikhomirova  
Altai State University, Russia

The initiation stage of *Iris ensata* Thunb. tissue cultures is optimized when flower fragments containing meristem tissue and with morphogenetic capacity are used. Direct regeneration of inflorescence axis (rachis) and perianth tube explants in tissue culture led to the formation of shoots typical of this species. Examination of the anatomical

structures of the ovary, pistil and filament did not show any zones of meristematic activity, as examined over a 30-d cultivation period using these organs. These results could explain the lack of regenerative capacity of these explants.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Phylogenetic polymorphism in neera-brahmi, *Bacopa monnieri* (L.) using molecular markers

Sanjoris Anu

Bios Research Centre, India

**B***acopa monnieri* (L.) Penn. commonly known as the neera-brahmi belongs to the family *Scrophulariaceae* is a small prostrate herb that grows wild in marshy and damp places near water logs, spreading throughout India. Over exploitation of this herbal plant species and the necessity of biodiversity conservation of this endangered plant species have attracted global attention. Accordingly, among the seven highly endangered identified plant species that need immediate attention, *B.monnieri* plants have a unique position as has been highlighted by NMPB, India and Technology Information Forecasting and Assessment Council (TIFAC), DST, Government of India (<http://www.nmpb.nic.in/prioritisedmedicinalplants.htm>). In this way biotechnologists also, took much interest in analyzing and monitoring the

systemic status of this valuable plant species. Identification of plant varieties/genotypes using modern molecular markers as PCR/RFLP profile have always been sought as reliable, inexpensive, easily obtainable and versatile set of molecular tools as repeatable DNA amplification sequence being relied upon in describing the accurate systematic status of any plant or animal species. The present study envisages tracing the genetic variations using their conserved genome like mitogenome and other different long and short primer polymorphic sequences, thereby intending to explore the population structure of *Bacopa monnieri* (L.) in India.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Genetic transformation of locally developed two cotton varieties [CRSP1 and CRSP2] with Bt and glyphosate tolerant genes

Shafique Ahmed

University of the Punjab, Pakistan

Expression of the transgene with a desirable character in crop plant is the ultimate goal of transgenic research. Transformation of two Bt genes namely Cry1Ac and Cry2A cloned as separate cassette under 35S promoter in pKHG4 plant expression vector was done by using shoot apex cut method of Agrobacterium. Molecular confirmation of putative transgenic cotton plants for Cry1Ac, Cry2A and GT gene was done through PCR and ELISA. Transformation efficiency of CRSP-1 and CRSP-2 was calculated to be 1.2 and 0.8% for Cry1Ac while 0.9 and 0.6% for Cry2A and 1.5 and 0.7% for GTG respectively. CRSP-1 was found to adopt natural environment (acclimatized) earlier than CRSP-2 when exposed to sunlight for one month. Expression of Cry1Ac, Cry2A and GTG was found to be 1.2, 1 and 1.3 ng/ $\mu$ l respectively for CRSP-1 as compared to CRSP-2 where expression was recorded to be 0.9, 0.5 and 0.9 ng/ $\mu$ l respectively. FISH analysis of the transgenic CRSP-1 and CRSP-2 demonstrated the presence of one and two copy

numbers respectively. Similarly, the response of CRSP-1 against Glyphosate @1900 ml/acre was far better with almost negligible necrotic spot and efficient growth after spray as compared to CRSP-2 where some plants were found to have necrosis and negative control where the complete decay of plant was observed after seven days of spray assay. Similarly, almost 100% mortality of 2nd instar larvae of *Heliothis armigera* was recorded after three days in CRSP-1 as compared to CRSP-2 where insect mortality was found to be less than 90%. Quantitatively speaking non transgenic plants were found with 23–90% leaf damage by insect, while CRSP-1 was with less than 5% and CRSP-2 with 17%. Taken together CRSP1 was found to have better insect control and weedicide resistance along with its natural ability of genetic modification and can be employed by the valuable farmers for better insect control and simultaneously for better production.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Analysis of genetically modified BT and cp4EPSPS cotton cultivars for transformation efficiency, acclimatization, expression and toxic levels to insects

Tahir Rehman Samiulla  
University of the Punjab, Pakistan

The major application of Biotechnology is the transfer of desirable characteristic in the host. To attain resistance against insects and weeds, this application was employed to transfer a double Bt and a glyphosate gene into two cotton varieties, FH-114 and CIM-598. Three genes Cry1Ac, Cry2A and Glyphosate gene were transferred through the Agrobacterium method using a plant expression vector with genes under the control of the CaMV35S promoter and NOS terminator sequence. Confirmation of insertion and expression of these genes in cotton plants was done through PCR and ELISA. Transformation efficiency for FH-114 and

CIM-598 was 1.2% and 0.8% for Cry1Ac, 0.9% and 0.6% for Cry2A and 1.5 and 0.7% for GTG respectively. FH-114 plants acclimatized better than CIM-598 plants when exposed to sunlight. Cry1Ac, Cry2A and GTG proteins were 1.2, 1 and 1.3 ng $\mu$ l<sup>-1</sup> for FH-114 which was more than CIM-598 for all three genes. FH-114 plants were able to control better insects and weed damage when subjected to a cotton leaf bioassay. Taken together, FH-114 genetic profile was more suitable for genetic modification to control insects and weed when compared to CIM-598.

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# ANNUAL BIOTECHNOLOGY CONGRESS

August 17-18, 2017 | Toronto, Canada

## Improvement of rice variety PAU 201 through marker assisted selection for grain color and bacterial blight resistance

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**P**AU 201, a very high yielding medium duration rice variety was released for cultivation in Punjab state in 2007, however, due to red pericarp color; the variety did not meet the specification of Food Corporation of India and was officially withdrawn in the year 2010 for cultivation in Punjab. Due to high yield potential, this variety covered more than 20% area in Punjab in two years after its release. In addition to red pericarp, this variety had only one bacterial blight resistance gene xa13. The red aleurone is due to single gene, designated as Rc7 and is located on chromosome 7. Both these genes are cloned and gene based primers have been designed. Due to consistent demand from farmers for improvement of this variety, we have improved this variety

through MAS by replacing Rc7 allele with recessive allele rc7, and additional bacterial blight resistance gene Xa21. A set of BC2F4 progenies selected for white grain color and bacterial blight resistance having more than 90% of the recurrent-parent genome were evaluated for yield and yield components. Lines that significantly out-yielded the recurrent parent and the check cultivars in station trials are evaluated at multiple locations in national-level nurseries for identifying the lines that could be released as varieties. These lines, in addition to being released as cultivars, can also be used as immediate donors for further improvement of rice cultivars.

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