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Conference Proceedings

Abstracts of invited lectures delivered during 2nd International Conference on Applied Biosciences (ICAB2021) held in Mohammad Ali Jinnah University, Karachi, Pakistan during December 30-31, 2021.

Preface

Department of Biosciences, Mohammad Ali Jinnah University, Karachi, Pakistan organized 2nd International Conference on Applied Biosciences (ICAB2021) during December 30-31, 2021. Hundreds of scientists, post-doctoral fellows and graduate students from Pakistan and other countries attended this conference. In this conference, an array of topics related to applied biosciences including Biochemistry, Molecular Biology, Bioinformatics, Metabolomics, Biotechnology, Cancer Biology, Genomics, Metagenomics, Proteomics, Health Biotechnology, Immunology, Infectious Diseases, Microbiology, Genetic Engineering, Molecular Medicine, Neurosciences, Virology were discussed.

In this issue of PJBMB, we are publishing abstracts of invited lectures delivered during the conference. All abstracts submitted to ICAB2021 for presentations in technical sessions were peer reviewed. Editorial board of PJBMB is grateful to the organizing committee of ICAB2021 for providing abstracts of invited presentations for publication in PJBMB.

Editorial Board

Pakistan Journal of Biochemistry and Molecular Biology

INVITED LECTURES IN ICAB21

THE EFFECT OF CYTARABINE ON MITOCHONDRIAL ACTIVITY OF KG1A CELL LINE

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ABSTRACT

Cancer is thought to be caused by the deformation of normal cells and their transformation into cancer cells. These cells proliferate uncontrollably and produce cells like their own. In this view, the basic idea is that every cancer cell is like any other cancer cell, but studies have shown that this view is wrong. New studies show that there are a number of cells in a cancerous mass that play a key role in cell proliferation. In fact, cancer cells also have cancer stem cells that are responsible for proliferation. On the other hand, it was known that the metabolism of cancerous cells differs from normal cells. In this study, we investigated the effect of the anticancer drug, Citarabin, on the mitochondrial activity of KG1a cell lines using MTT, OXPHOS, and glycolysis inhibitors.

HOST AND SARS-COV-2 GENETIC VARIATIONS

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ABSTRACT

In the two years of pandemic, SARS-CoV-2 has claimed over 5.2 million lives with total infection numbers raised more than 264 million. The causative agent of the pandemic, SARS-CoV-2, is not completely new to us, since an earlier variant has caused relatively limited epidemic in 2003. Later from 2008-2012 another variant, MERS, established another epidemic of limited scale. However, this time, evolutionary forces works in favour of the virus and resulted in an emergence of a new form of the etiological agent that appeared as rather more formidable contagion than antecedent strains. Nevertheless, the interplay of the evolution is still going on and both host(s) and the virus, SARS-CoV-2, are exploiting their ingrained and/or newly emerged genetic variations in a battle of survival. This talk is the discussion in relation to the findings in this connection undertaken at Dow University of Health Sciences.

ADSORPTION OF HEXAVALENT CHROMIUM USING NANO MAGNETIZED ACTIVATED CARBON PRODUCED FROM FILAMENTOUS ALGAE

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ABSTRACT

Due to the toxic and dangerous properties of hexavalent chromium (VI (Cr)), its removal by an efficient method is necessary. The use of activated carbon prepared from filaments algae as an adsorbent is a promise method in the removal of hexavalent chromium. The aim of this study was to produce magnetized activated carbon from filaments algae and investigation of its Hexavalent chromium removal efficiency aqueous solution. For investigation of the characteristics of synthesized nanomaterial, the different analyses, including XRD, FE-SEM, and VSM were down. Isotherm and kinetic models were carried out for determination of adsorption constant. Results showed that there is a suitable correlation between the values obtained by experiments and the values predicted by the quadratic model ($P < 0.05$). Results revealed that the efficiency of Cr (VI) adsorption decreases by an increase in pH values and an increase in the reaction time and adsorbent dosage was led to development of the process efficiency. The optimum conditions were pH of 3, Cr (VI) concentration of 40 mg/L, the adsorbent concentration of 1 g/L, and time of 90 min. At these determined conditions, Cr (VI) adsorption efficiency was higher than 21.90 %. The Cr (VI) adsorption experimental data were superlatively fitted to the Freundlich isotherm model and the removal followed the pseudo-second-order kinetic model (R2 >0.99). The experimentally obtained maximum adsorption capacity of AC-Fe3O4-NPs for Cr (VI) adsorption was found to be 15.24 mg/g.

Keywords: Activated carbon, Adsorption, Hexavalent chromium

EXPLORING SALIVARY PROTEINS IN DENTAL CARIES AND FOR SYSTEMIC MARKER PROTEIN

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ABSTRACT

In recent years, human saliva has emerged with lots of potential to be used as a diagnostic tool that can revolutionize the next generation diagnostics in a way that is safe, easy, inexpensive and non-invasive. This has become possible because of the molecules such as proteins and nucleic acid that are present in saliva and can reflect the physiology of an individual. In the earlier period saliva was mainly studied for its role in the oral cavity rather than in assessing the systemic diseases. With the advancements in technology and tools in molecular research, the role of saliva as a bio-fluid for the diagnosis of disorders has been surfaced with strong impact. We present here the study conducted on dental caries and myocardial infarction patients utilizing saliva samples. In dental caries the differentially expressed proteins were observed and evaluated. These include proteases, IgA and other protein constituents of saliva. Similarly the saliva collected from myocardial infarction patients to evaluate the presence of cardiac troponin I presented a moderate positive correlation as compared to serum cardiac troponin I levels.

Keywords: Saliva, Dental Caries, Myocardial Infarction

PRODUCTION, CHARACTERIZATION AND PILOT SCALE APPLICATIONS OF A PROTEASE FROM BACILLUS SUBTILIS ZMS-2 IN LEATHER PROCESSING

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ABSTRACT

Proteolytic enzymes are highly desirable for various commercial processes including leather processing, diagnostics, detergent formulations and waste management. Current study focuses on the scale up production of a protease from Bacillus subtilis ZMS-2 using statistically optimized production medium followed by its pilot scale application as dehairing and bating agent. The optimum time and concentration required for dehairing was 90 min and 1.5% (460 U mL-1), respectively, using 2% sulfide and 3% lime. Enzymatically processed skins exhibited better physical properties than control including tensile strength (16.35±6.68N/mm), ball burst (452.88±6.06N/mm), % elongation (38.85±1.06N), tear strength (50.16±4.42N/mm) and softness (6.5mm). Electron microscopy of skin reveled complete removal of hairs with roots, confirming the keratin specificity of enzyme. Moreover, enzymatic process reduced the Chemical Oxygen Demand (COD), Bio-chemical Oxygen Demand (BOD), Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) by 68, 77, 34 and 39%, respectively. The protease from B. subtilis ZMS-2 can be concluded as a potential dehairing and bating alternative for the eco-friendly processing of animal skins at commercial scale.

Keywords: Bacillus subtilis ZMS-2; pilot scale; dehairing; bating; eco-friendly.

CHANGING THE TREATMENT PARADIGM; TREATING THE VIRUS BY TARGETING THE CELL SIGNALING

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ABSTRACT

Our lab in the Department of Molecular Genetics, University of Toronto, Toronto, Canada has worked on anti-viral drug discovery for many years. We are interested in targeting the host proteins/genes as this approach will not only help us in overcoming the drug resistance problem but will also help identify common druggable targets for different viruses. We have screened hundreds of small molecules and our results show that some of these compounds not only exhibit broad-spectrum anti-viral activity but also are well tolerated in the mammalian cell culture system. Several such compounds inhibit wild-type and drug-resistant HIV-1 strains by preventing expression of several HIV-1 proteins including Gag and Env. The initial results showed that the antiviral activity of one of these compounds is exerted by the activation of GPCRs receptors followed by MEK1/2-ERK1/2 signaling. We have developed different assays and virus-specific mammalian cell culture systems to not only test the drug toxicity, but also the IC50 of the drugs against the viruses. Here we are presenting the initial antiviral and toxicity results, and our strategy to identify/characterize host drug targets and the potential pathways/protein/genes involved in the function of antiviral drug candidates. Although previous studies in the lab have identified few proteins/genes involved in the drug action pathways, we are interested in identifying other cellular proteins and hopefully the complete pathways helping the antiviral activities of these compounds. These studies will not only add to the existing knowledge and understanding of viral pathogenesis but will also help in designing more effective treatment in future.

Keywords: HIV, Anti-viral, GPCRs, HIV Splicing,

MATRIX METALLOPROTEINASES AND AUTOIMMUNE THYROIDITIS

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ABSTRACT

Hashimoto's Thyroiditis (HT), being the most common type of autoimmune thyroiditis (AIT), acts as the leading cause of hypothyroidism. To date, it has been found that the development of the disease is due to a combination of genetic factors and environmental factors. One of the most important pathogenetic mechanisms of AIT is the violation of immunological tolerance and the development of the autoimmune process, the markers of which are various biologically active substances, in particular, matrix metalloproteinases (MMP) of the extracellular matrix (ECM). The purpose of the work is to study the activity of key metalloproteinases and the level of macroglobulin α2-MG in patients with autoimmune thyroiditis.. In 2014-2020, a single-center open prospective non-

randomized study was conducted, which conducted a complex examination of 170 patients with autoimmune thyroiditis, 64 men and 106 women, aged 18 to 64 years. The control group was 65 people without TG diseases and autoimmune pathology aged 20 to 65 years, of which 26 were men and 39 were women. The diagnosis of the subclinical form of AT was made based on an increase in TSH level in the background of normal levels of hormones T3 and T4 in blood plasma. This form of the disease was characterized, as a rule, by an erased clinical picture. In patients of all groups, the activity of MMP-3 and 7 was determined by solid-phase enzyme immunoassay, «Biosource» kits (Belgium) were used to determine the level of MMP-3, and «Quantikine» reagents (USA) were used to evaluate the activity of MMP-7. The level of α2-MG in patients with AIT was evaluated by an immunoturbidimetric method, the principle of which is based on the reaction between alpha-2MG and polyclonal antiserum in the presence of polyethylene glycol, used reagents from «Sentinel» (Italy). The study of matrix metalloprotein activity in the examined patients showed a statistically significant ($p = 0.015$) increase in MMP-3 and MMP-7 activity in patients with AIT compared to the corresponding parameters in persons of the control group. Thus, levels of MMP-3 and 7 were in the group of patients, respectively 56 (51.0; 59.0) and 4.6 (4.3; 5.2) ng/ml, in control 23.0 (16.0; 26.0) and 3.6 (3.4; 4.1) ng/ml, respectively. At the same time, there were no statistically significant intergroup differences in the MMP-7 indicator in patients with different forms of hypothyroidism. At the same time, in both subclinical and manifest forms of the disease, activity levels MMP-3 and 7 were significantly higher than levels of similar indicators in the control group. The obtained results indicated that the degree of increase in MMP activity in the blood serum of patients with hypothyroidism can vary significantly. Differences in MMP-3 and α2-MG activity in groups of patients with different clinical forms of hypothyroidism were less pronounced.

Keywords: Hashimoto's Thyroiditis (HT), autoimmune thyroiditis (AIT), matrix metalloproteinases (MMP), α2 macroglobulins (α2-MG)

THERAPEUTIC POTENTIAL OF SCORPION VENOM

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ABSTRACT

Pakistan is a dreamland for venom researchers. Venomous fauna of any country provide plethora of template for dug designing. Various pain killers, anticancer, antimalarial, antidiabetic, antiplatelet, antihypertensive compounds etc. have been discovered from venom using proteomics and computation tools. Many of these compounds are either approved by FDA (e.g., Captopril) or in clinical trial (e.g., Chlorotoxin). The objective of this study was to explore the medicinal potential of Pakistani scorpion (Buthus Sindicus) venom. The venom was collected by gentle tapping and /or electrical stimulation of telson. After protein estimation the size exclusion chromatography (SEC) and HPLC was used

for purification of peptides. The toxin named as Bs-KTx6 (4,115.4 Da) has been isolated, synthesized and found to be a potent as well as selective inhibitor of voltage gated potassium channel Kv1.3 (IC50 = 7.7 pM). The NMR structure of Bs-KTx6 revealed the presence of βαββ scaffold as predicted in homology model. The docking studies of Bs-KTx6 with potassium channels as well as patch clamping studies shows that, this toxin could be used as a template in designing drugs for the treatment of autoimmune diseases. **Keywords:** Scorpion, Toxins, Potassium channel, venom

URSOLIC ACID AND ITS AMIDE DERIVATIVES DISRUPTS CLINICAL ACINETOBACTER BAUMANNII ISOLATES

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ABSTRACT

Hospital acquired infections due to antimicrobial resistant pathogens has emerged globally with increased morbidity and mortality. A group of ESKAPE pathogens holds a key importance in hospital acquired infections. Acinetobacter baumannii is one of the members of ESKAPE pathogens, its spreading widely and acquired multiple drug resistance (MDR) even with the last resort drug colistin at rapid phase which posed an aptitude of problem in term of its treatment or management. World Health Organization (WHO) categorized A. baumannii among the list of pathogens for which new pharmacophore required on urgent basis. Compounds from medicinal plants and their derivative might act as potential drug candidates for MDR pathogens. So, in this study Ursolic acid (UA) and its synthetic amide derivatives were screened against standard (ATCC: 19606) and clinical isolates of A. baumannii strains. In the first phase of this study clinical isolates were collected and identified as A. baumannii strains phenotypically as well as genotypically. Then the ursolic acid and its derivatives were screened for antimicrobial, biofilm inhibiting and eradicating potential. Out of tested compounds amide derivative of UA (KSUA-2,4) was found to possess better antimicrobial concentration at 77.87µg/ml against colistin resistant A. baumannii strains (Colistin MIC > 100µg/ml). Compound KSUA-2,4 significantly inhibited or eradicated >60% biofilm formation of tested standard and clinical isolates at MIC. Microscopic analysis further confirms the biofilm inhibition and eradication potential of this compounds. Atomic Force Microscope analysis (AFM) further confirms the antimicrobial properties KSUA-2,4 and suggesting that the antimicrobial action might be due the the membrane leakage. Considering this evidence, microbial membrane potential was determined by using FACS analysis which confirm the loss of membrane potential after compound treatment. Gene expression analysis further explained that this compound inhibits biofilm formation by reducing the gene expressionof bap (biofilm gene) and abaR (quorum sensing). So, ursolic acid amide derivative KSUA-2,4 might be used to tackle Acinetobacter baumannii related nosocomial infections and further evaluated as a drug candidate.

FUNCTIONAL VARIANTS IN COMPLEMENT GENES ARE ASSOCIATED WITH GENETIC SUSCEPTIBILITY TO LEPROSY

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ABSTRACT

Leprosy is a chronic infectious disease caused by the intracellular bacterium Mycobacterium leprae. It is predicted that globally two to three million people are disabled permanently because of leprosy. There is a large body of evidence indicating a crucial role for the host genetic background in disease outcome. Previous genetic studies have identified a variety of risk loci and/or susceptibility genes for leprosy. The complement system is an integral part of innate immunity and plays an important role in host immunity to prevent leprosy infection. The association of complement components with the genetic susceptibility of leprosy have not been sufficiently studied. In this study, we systematically analyzed the genetic variants and mRNA expression patterns of 22 complement system genes through targeted NGS and mRNA expression profiling. We have validated the association of the previously identified FCN2 with susceptibility to leprosy (rs7851696, c.772G>T, p. Ala258Ser, P-value = 2.5×10-6, OR = 0.655). Missense mutations in the complement core component C3 (rs2277984, P-value = 2.4×10-5, OR = 1.491), complement receptor CR1 (rs2274567, c. 4973A>G, p. His1208Arg; P = 6.4□10-6, OR = 1.381, rs41274776, c. *37A>G, P = 2.1□10-5, OR = 1.35, rs3811381, c. Pro1827Arg; P = 3.4□10-5, OR = 1.348, and rs6691117, c. 6193A>G, p.Ile1615Val; P = 3.6□10-5, OR = 1.342) and CR1L (rs3085, c. 415A>G, p. Ile139Val; P = 6.7□10-10, OR = 1.527, and rs2296158, c. 346A>G, p. Arg116Gal; P = 1.4□10-5, OR = 1.393) were associated with the genetic risk of leprosy. Additionally, we found an alteration in mRNA expression of these genes in M. leprae infected cells and skin lesion of leprosy patient. In summary, we found that genetic variants in FCN2, C3, CR1, and CR1L are associated with leprosy. These results indicated that leprosy is genetically determined and provided a basis for understanding this ancient infectious disease.

Keywords: Leprosy, Complement System, Functional variant, Missense variant

CONSTRUCTION OF FUSION ANTIGENS OF MYCOBACTERIUM TUBERCULOSIS FOR DIAGNOSTIC AND THERAPEUTIC APPLICATIONS

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ABSTRACT

Tuberculosis (TB) is amongst the deadliest diseases worldwide. For effective control of TB a rapid, reliable and sensitive method for its diagnosis as well as improved treatment therapies are essential. Fusion proteins based on antigens of Mycobacterium tuberculosis (Mtb) can be exploited to develop a

serodiagnostic procedure which can meet the requirement of reliability, high throughput and cost effectiveness. Thus, two truncated variants of Mtb antigen Rv1984c, i.e, Tn1Rv1984c and Tn2Rv1984c were expressed in Escherichia coli. In Tn1Rv1984c, 47 amino acids from the N-terminus and 27 amino acids from the C-terminus were removed, while Tn2Rv1984c had 130 amino acids removed from the N-terminus. Screening of Rv1984c, Tn1Rv1984c and Tn2Rv1984c against 231 sera samples from the culture positive TB patients showed sensitivities of 34.2%, 49.4% and 26.8%, respectively. Another antigen Rv1352 was analyzed for the location of epitopes, which were not reported before. The fusion molecule i.e. Tn1Rv1984c-Rv1352 was designed and expressed in E. coli which showed the sensitivity of 62.8%. By joining another antigen, Rv2031c, to the N-termini of the fusion, sensitivity of the Rv2031c-Tn1Rv1984c-Rv1352 was enhanced to 71.4%. The fusion construct Rv2031c-Tn1Rv1984c- Rv1352 showed comparatively higher sensitivity of 73.4% in the male group as compared to 67% in the female group. Rv2031c-Tn1Rv1984c-Rv1352 also showed comparatively higher sensitivity of 78.1% in the patients of 16-35 years, which is considered more vulnerable age group. Vaccine potential of all the single and fusion proteins was also evaluated on the basis of IFN-γ produced in the human PBMCs. Average values of IFN-γ released by incubation of the isolated human PBMCs with the antigens i.e. Rv2031c, Rv1984c, Tn1Rv1984c, Rv1352, Tn1Rv1984c-Rv1352 and Rv2031c-Tn1Rv1984c-Rv1352 were 83.0, 63.4, 68.7, 77.1, 153.4 and 267.6 pg IFN-γ released per mL of the culture containing 2.5X105 cells. Data derived for the secondary structure analysis through Circular Dichroism (CD) spectroscopy and prediction on the basis of molecular modelling were also in agreement. Thus, Rv2031c-Tn1Rv1984c-Rv1352 can be a potential base for producing constructs showing good therapeutic potential as well as greater diagnostic sensitivity through fusion of epitopes from additional Mtb antigens.

Keywords: Tuberculosis, Tn1Rv1984c, Novel epitope prediction for Rv1352. Rv2031c-Tn1Rv1984c-Rv1352, Mtb-specific antibody detection. IFN-γ release estimation.

ENGINEERING STEM CELLS; AN APPROACH TO CURE PAIN AND INFLAMMATION IN SPINAL CORD

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ABSTRACT

Introduction: Low back pain (LPB) is the main cause of disability worldwide with enormous socioeconomic burdens. A major cause of LBP is intervertebral disc degeneration (IVDD): a chronic, progressive process associated with exhaustion of the resident cell population, tissue inflammation, degradation of the extracellular matrix and dehydration of the nucleus pulposus. The current standard of care is limited to symptomatic relief and no current approved therapy promotes disc regeneration. However, no treatment is actually able to treat IDD and hamper the degenerative

process. Therefore, the intradiscal injection of stem cells is raising as a promising approach to regenerate the IVD. Objective: The objective of the current study was to differentiate hUC-MSCs into chondrocytes by overexpressing chondrogenic transcription factors, Sox9, and Six1 and their combination (Sox9+Six1), in vitro. Following their transplantation into rat model of degenerated intervertebral discs, to elucidate their regenerative potential and capability to reduce pain and inflammatory profile in comparison to induced chondro-proginator cells (iCPCs) and hUC-MSCs. Results: hUC-MSCs were isolated and characterized morphologically and immunologically by expression of specific markers. hUC-MSCs were transfected with mammalian expression vectors sox-9 and six-1 transfection factors and were assessed for chondrogenic lineage based on expression of specific markers. These differentiated MSCs were implanted in the rat model of IVDD. The regenerative potential of implanted cells was investigated using biochemical and structural analysis of IVDs. Transcriptional analysis was done to assessed the anti-inflammatory and pain profile of IVDD. Conclusion: Genetically modified hUC-MSCs accelerated the cartilage regeneration and proved to be a promising therapeutic strategy due to their immuno-modulatory and anti-inflammatory properties significantly downregulated pain and inflammatory profile. which provides a unique opportunity and impetus for stem cell-based based therapeutic approach for degenerative disc diseases.

Keywords: Intervertebral disc degeneration, Transcriptional factor, Mesenchymal stem cells, Pain and inflammation, Regeneration.

IMPROVING RICE CROP YIELD BY MINING NATURAL VARIATIONS OF NPQ DYNAMICS BY GWAS

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ABSTRACT

With the increase in the global population and limited arable land area, there is an urgent demand to improve crop yield. Improvement in photosynthetic efficiency is regarded as a major option to overcome this phenomena. Photosynthesis needs light; but when there is too much light, photosynthesis can be inhibited or damaged. The saturation maxima for photosynthetic productivity is achieved at far lower than the maximum sunlight at most time of the day. Non-photochemical quenching (NPQ) is a mechanism used by plants to cope with excess light. However, NPQ and photosynthesis have a very complex relationship, NPQ continuously competing photosynthesis, usually, this competition is beneficial for the plants, especially under fluctuating light. However, at some instant NPQ over-influences photosynthesis and drain the possible captured power that can drive electron from PSII in the form of heat. The goal of this study is to identify genetic architecture controlling NPQ. Therefore, natural variation of NPQ dynamic traits were assessed using a global rice mini-core panel (RMCP) comprising of 199 rice accessions.

Our results show that NPQ magnitude trait was 21% higher in the Japonica (JAP) rice varieties compared to the Indica (IND) subpopulation. Similarly, the JAP population showed faster NPQ induction and NPQ relaxation kinetics compared to the IND population. Genetic analysis showed that NPQ parameters are under strong genetic control. Later on, we conducted Genome Wide Association Studies (GWAS) analysis of NPQ related parameters in the RMCP. GWAS results reveal that plastid-associated lipid protein (OsPAP4) was strongly associated with the NPQ magnitude trait. We found four SNPs extending over the promoter and CDS regions of OsPAP4 with significant p values and resulting 4 haplotypes. The haplotype I was mostly found in JAP varieties and was associated with high NPQ magnitude while haplotype III was mostly present in AUS accessions and associated with low NPQ magnitude. Subcellular localization results confirm that OsPAP4 is in the chloroplast. Furthermore, knockout rice lines of Ospap4 showed higher NPQ magnitude with a decreased plant growth and decreased CO2 assimilation rate compared to WT. In contrast, the OsPAP4 overexpression rice lines showed an opposite trend. The results here demonstrate that the OsPAP4 is a gene controlling NPQ magnitude, and hence can be a potential target to manipulate to gain increase photosynthesis and biomass in the field. With the increase in the global population and limited arable land area, there is an urgent demand to improve crop yield. Improvement in photosynthetic efficiency is regarded as a major option to overcome this phenomena. Photosynthesis needs light; but when there is too much light, photosynthesis can be inhibited or damaged. The saturation maxima for photosynthetic productivity is achieved at far lower than the maximum sunlight at most time of the day. Non-photochemical quenching (NPQ) is a mechanism used by plants to cope with excess light. However, NPQ and photosynthesis have a very complex relationship, NPQ continuously competing photosynthesis, usually, this competition is beneficial for the plants, especially under fluctuating light. However, at some instant NPQ over-influences photosynthesis and drain the possible captured power that can drive electron from PSII in the form of heat. The goal of this study is to identify genetic architecture controlling NPQ. Therefore, natural variation of NPQ dynamic traits were assessed using a global rice mini-core panel (RMCP) comprising of 199 rice accessions.

Our results show that NPQ magnitude trait was 21% higher in the Japonica (JAP) rice varieties compared to the Indica (IND) subpopulation. Similarly, the JAP population showed faster NPQ induction and NPQ relaxation kinetics compared to the IND population. Genetic analysis showed that NPQ parameters are under strong genetic control. Later on, we conducted Genome Wide Association Studies (GWAS) analysis of NPQ related parameters in the RMCP. GWAS results reveal that plastid-associated lipid protein (OsPAP4) was strongly associated with the NPQ magnitude trait. We found four SNPs extending over the promoter and CDS regions of OsPAP4 with significant p values and resulting 4 haplotypes. The haplotype I was mostly found in JAP varieties and was associated with high NPQ magnitude while haplotype III was mostly present in AUS accessions and associated with low NPQ magnitude. Subcellular localization results confirm that OsPAP4 is in the chloroplast. Furthermore, knockout rice lines of Ospap4 showed higher NPQ magnitude with a decreased plant growth and

decreased CO2 assimilation rate compared to WT. In contrast, the OsPAP4 overexpression rice lines showed an opposite trend. The results here demonstrate that the OsPAP4 is a gene controlling NPQ magnitude, and hence can be a potential target to manipulate to gain increase photosynthesis and biomass in the field.

Keywords: Photosynthesis, Non photochemical quenching (NPQ), Genome wide association studies (GWAS), Plastid-Associated Lipid Protein (OsPAP4)

COMPARATIVE STUDIES ON GENE COMPOSITION AND ORDER IN GENOMES OF POMEGRANATE GULOYSHA, DABENZI AND TUNISIA VARIETIES

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ABSTRACT

In 2017-2019, the nuclear genomes of four different varieties of pomegranate (Punica granatum), Azerbaijan Guloysha, Dabenzi, Tunisia and Taishanhong) were sequenced. In this study, the gene composition of the nuclear genomes of three varieties, Azerbaijan Guloysha (https://www.ncbi.nlm.nih.gov/genome/13946?genome_assembly_id=357360), Dabenzi (https://www.ncbi.nlm.nih.gov/genome/13946?genome_assembly_id=322009) and Tunisia (https://www.ncbi.nlm.nih.gov/genome/13946?genome_assembly_id=720008) with available genome annotation were studied and the results were compared with similar data on Arabidopsis thaliana, Eucalyptus grandis, Malus domestica, Prunus persica, Rubus occidentalis and Solanum lycopersicum, Oryza sativa (Japonica and Indica) reference genomes. It was found that with at least 60% similarity throughout the complete protein sequences, most of the 50,476 proteins of the Guloysha pomegranate variety have numerous homologues in the Dabenzi and Tunisian pomegranate varieties, as well as in eucalyptus, Arabidopsis, peach, black raspberry, apple and tomato. Results obtained indicate that Guloysha is much closer to Tunisia than Dabenzi. Moreover, thousands of proteins of each of the three varieties of pomegranate could not be found in other pomegranate varieties. While some of these differences are thought to be due to the genome sequencing gaps and annotations errors, it is possible that there may be some real differences between the protein sets of different varieties of P. granatum. Approximately half of the immediate neighbour gene pairs in the genome of each pomegranate variety are organized in the "Tail-Head" fashion. Homologues of only 10-25% of the gene pairs of Dabenzi and Tunisian varieties have the same arrangement (B-B or Q-B or Q-Q) in the Guloysha variety. It seems that after a divergence from the common ancestor, there were significant rearrangements in the organization of the genomes of these three pomegranate varieties.

Keywords: pomegranate; genome; protein genes; Head-to-Head genes; Tail-to-Head genes; Tail-to-Tail genes; intra-species comparison.