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Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.

Yours sincerely,

Vasco Azevedo

Vasco Azevedo, Editor-in-Chief
Integrative Omics and Applied Biotechnology
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CONGRATULATIONS, ACKNOWLEDGEMENTS, AND INSPIRATIONS

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EDITORIAL

The Editorial refers to the Nobel Prizes in Physics, Chemistry and Medicine/Physiology awarded in 2012. Here we would like to shortly celebrate the achievements of brilliant scientists but also rejoice in the success of basic science research that led to significant technological and medical applications.

On behalf of the ***Institute of Integrative Omics and Applied Biotechnology*** (IIOAB) and of the Editorial Board of ***The IIOAB Journal***, we would like to congratulate Serge Haroche and David J Wineland, Brian K Kobilka and Robert J Lefkowitz, John B Gurdon and Shinya Yamanaka, on their respective Nobel Prizes in Physics, Chemistry and Medicine/Physiology awarded in 2012.

2012 Physics Laureates: David J. Wineland (left) and Serge Haroche (right) during their interview with Nobelprize.org. Copyright © Nobel Media AB 2012; Photographer: Niklas Elmehed.

Serge Haroche was born in Morocco in 1944. He is a Professor at the College de France. **David J. Wineland** was born in Milwaukee in 1944 as well and graduated in California. He is a physicist at the National Institute of Standards and Technology. Both eminent researchers have been recognized for their "ground-breaking experimental methods that enable measuring and manipulation of individual quantum systems". Serge Haroche made a huge contribution to Cavity Quantum Electrodynamics to study single atom spontaneous emission enhancement, decoherence of state superpositions and atom-photon entanglement [1]. His team was able to store photons between mirrors for "long" periods of time allowing a non-destructive method of detection. His ideas have paved the way to build new devices for opto-electronics and optical communication science. David Wineland first achievement was the establishment of laser cooling that has been used to trap ions and test theories in quantum physics, such as entanglement with two and four ions [2]. The applications of his discoveries are enormous. His research led to the construction of a quantum logic atomic clock, the world's most precise clock, and to the basis of building super fast large-scale quantum computers.

The American physician and scientist **Robert J. Lefkowitz** was born in 1943. He is a Professor of Medicine and Professor of Biochemistry at Duke University. **Brian K. Kobilka** was born in 1955 in Minnesota and is a Professor of Molecular and Cellular Physiology at Stanford University. He worked as a postdoctoral research fellow under Lefkowitz's supervision. They have been awarded for their remarkable contribution to the investigation of the structure and function of G protein-coupled receptors and to the understanding of the role and regulation of these receptors. Robert Lefkowitz is one of the fathers of receptor biology and is well known for his work on the sequence, structure and function of beta-adrenergic and related receptors. He also discovered and characterized two families of regulatory proteins: G-protein coupled receptor kinases (GRKs) and β-arrestins [3].

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2012 Chemistry Laureate Robert J. Lefkowitz. Copyright © Nobel Foundation 2012. Photographer: Ulla Montan.

2012 Chemistry Laureate: Brian K. Kobilka. Copyright © Nobel Foundation 2012. Photographer: Ulla Montan.

Brian Kobilka has a great interest in the biochemical and biophysical approaches allowing the characterization of the dynamic behaviour of G protein-coupled receptors. He obtained the first crystal structures of a hormone/neurotransmitter-activated GPCR. Further important receptor structures were later described, and recently three-dimensional images of GPCR bound to their ligands (agonists and antagonists) have been published [4].

The discovery that all G protein-coupled receptors share a similar molecular structure with seven transmembrane domains helped scientists from pharmaceutical industries to design potent compounds and target one of the largest protein families in humans (about 800 GPCRs have been identified so far). Nowadays, about 40 percent of all drugs prescribed are designed to target these receptors, including antipsychotics, antihistamines, ulcer drugs and beta blockers that treat cardiovascular diseases.

2012 Medicine Laureates: Sir John B. Gurdon (left) and Shinya Yamanaka (right) during their interview with Nobelprize.org. Copyright © Nobel Media AB 2012, Photographer: Niklas Elmehed.

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John B. Gurdon was born in 1933 and is an Emeritus Professor in the Department of Zoology at the University of Cambridge. **Shinya Yamanaka** was born in Higashiōsaka in 1962. He serves as a Professor at Kyoto University and as a senior investigator at the Gladstone Institute of Cardiovascular Disease. Their respective work on somatic cell nuclear reprogramming [5] and human pluripotent stem cells [6] led them to obtain the Nobel Prize this year. The initial and revolutionary experiment by John Gurdon in 1962 demonstrated that an immature cell nucleus in a frog egg cell could be replaced with the nucleus from a mature intestinal epithelium cell, and then developed into a tadpole. This laid the foundation for cloning experiments. Then a major step forward was brought about by Shinya Yamanaka who overcame the critical issue of working with cells derived from live human embryos. Indeed, it is since 2007 possible to turn adult somatic cells (from animals and now humans) into pluripotent stem cells and (re)program them into specialized cells such as neurones and cardiac myocytes. Fibroblasts from patients affected by diseases can therefore be reprogrammed into particular cell types in order to study them in vitro. This opens the perspective of an ethical regenerative medicine and will encourage the progress in personalized medicine.

All these impressive stories of determination, diligence, motivation, patience, humility, innovation, optimism and courage are the best illustrations that basic science should be further promoted and properly funded. Fundamental research thus works hand in hand with technology, medicine and social interests. It is still worth investing in these interconnections, both financially and humanly. In these difficult times of economical instability, it is now the right moment to think about how our resources should be stirred up to ensure a sustainable world.

We may take this opportunity to introduce here a new scheme in The IIOAB Journal. Each year the Editorial Board will select among the contributions published in the journal the article with the highest impact that will be recognized with the Best Article Award.

“Not everything that can be counted counts, and not everything that counts can be counted.”…….Citation attributed to Albert Einstein.

"I believe he has ideas about becoming a Scientist; on his present showing this is quite ridiculous."…….2012 Nobel Prize winner John Gurdon's school report card.

The opinions expressed in this article are not necessarily those of the Editors of The IIOAB Journal or of the Institute of Integrative Omics and Applied Biotechnology.

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CONFLICT OF INTERESTS

The author states that he has no conflict of interest pertaining to this manuscript.

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Blum et al.

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COMENTARY | OPEN ACCESS

NEUROGENETIC IMPAIRMENTS OF BRAIN REWARD CIRCUITRY LINKS TO REWARD DEFICIENCY SYNDROME (RDS) AS EVIDENCED BY GENETIC ADDICTION RISK SCORE (GARS): A CASE STUDY

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ABSTRACT

Importantly, research from our laboratory in both in-patient and outpatient facilities utilizing the Comprehensive Analysis of Reported Drugs (CARD)™ found a significant lack of compliance to prescribed treatment medications and a lack of abstinence from drugs of abuse during active recovery. This unpublished, ongoing research provides an impetus to develop accurate genetic diagnosis and holistic approaches that will safely activate brain reward circuitry in the mesolimbic dopamine system. Our laboratory has extensively published the neurogentics of brain reward systems with particular reference to genes related to dopaminergic function. In 1996, we coined "Reward Deficiency Syndrome" (RDS), used to describe behaviors found to have an association with gene-based hypodopaminergic function. Many subsequent studies have embraced RDS as a useful concept to help expand our understanding of Substance Use Disorder (SUD), process addictions, and other obsessive, compulsive and impulsive behaviors. Here, we illustrate the usefulness of the genetic testing of a panel of reward-related genes, the Genetic Addiction Risk Score (GARS) in only one case study. Interestingly, we were able to describe lifetime RDS behaviors in a recovery addict (17 years sober) blindly by just assessing resultant GARS data. We encourage further required studies in this important emerging field.

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KEY WORDS

Genetic Addiction Risk Score (GARS), Dopaminergic System, Reward Genes; Reward Deficiency Syndrome (RDS)

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[I] INTRODUCTION

The brain's mesolimbic reward system is a critical site for experiences of well-being. The reward center is where chemical messengers including serotonin, enkephalin, γ-aminobutyric acid (GABA), dopamine (DA), acetylcholine (ACH) and many second messenger proteins act in concert to provide a net release of DA in the nucleus accumbens (NAc). The idea that the synthesis, vesicular storage, metabolism, receptor formation, and catabolism of neurotransmitters are controlled by genes is well documented [1-3].

Most importantly, polymorphisms of reward genes can disrupt the neurochemical events that culminate in neuronal release of DA within the mesolimbic reward circuitry. A breakdown of these neuronal events in the "The Brain Reward Cascade" [4] will eventually lead to DA dysfunction. DA neurotransmission is essential for an individual to experience of pleasure (reward) and the reduction of stress. DA dysfunction then can result in a deficiency in reward and a predisposition to substance-seeking in an attempt to ameliorate hypodopaminergic function [5].

1.1. Neurogenetic considerations

Homo sapiens have a biological predisposition to drink, eat, reproduce, and desire pleasurable experiences. DNA polymorphisms, together with epigenetic and/or environmental factors can result in multiple impulsive, compulsive, and addictive behaviors by impairment of the normal flow of

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neurotransmitter activity in the reward center of the brain. From the many genes known to predispose individuals to excessive cravings and result in substance use disorders (SUDs), some of the most prominent genes with known polymorphisms make up the provisional GARS panel they include: the serotonergic 2A receptor (5-HTT2a); serotonin transporter (5HTTLPR); DA D1 receptor (DRD1); DA D2 receptor (DRD2); DA D3 receptor (DRD3); DA D4 receptor (DRD4); DA transporter (DAT1), and the catechol-O-methyltransferase (COMT), monoamine oxidase (MOA); Mu-opiate receptor (MOR); GABA –B3; Gamma 2 subunit genes; as well as the PENK Cytochrome P450 gene [5-7][Table–1].

Table: 1. Proposed Genetic Addiction Risk Score (GARS)

Dopamine D1 Receptor Gene
Dopamine D2 Receptor Gene
Dopamine D3 Receptor Gene
Dopamine D4 Receptor Gene
Dopamine D4 Receptor Gene
Serotonin 2a Receptor Gene
Serotonin Transporter Gene
Mu-opiate Receptor Gene
GABA –B_3 Receptor Gene
PENK Gene
Mono-Amine –Oxidase A Gene
Catecholamine –Methyl-Transferase Gene
Cytochrome P450 Gene

The first controversial study on the association of polymorphisms of the DRD2 A1 allele and severe alcoholism [4] started, the explosive field known as "Psychiatric Genetics". Since then an association has been identified between common genetic variants of the DA D2 receptor gene (DRD2) polymorphisms [8, 9] and other reward genes and polymorphisms [5, 6, 7] that result in hypodopaminergic function. An association between hypodopaminergic function and impulsive, compulsive, and addictive behaviors and has also been identified [5, 6, 10].

Individuals are predisposed to self-medicate with substances and behaviors that will trigger the release of DA. For example, an increased rate of mitochondrial DA breakdown due to increased MOA activity or an increased rate of synaptic DA breakdown due to having high catabolic genotype of the COMT gene lead to a "hypodopaminergic" trait. On the other hand, slower breakdown of DA due to polymorphisms in both the MOA and or COMT may lead to hyperactivity as seen in Attention Deficit Hyperactivity Disorder (ADHD).

Addictions, including alcohol, opiates, psychostimulants (cocaine, methamphetamine), nicotine, glucose, gambling, sex addiction, excessive spending, and even uncontrolled internet gaming are associated with the release of DA in the mesocorticolimbic system or reward pathway of the brain [4, 5, 11-14]. While activation of this dopaminergic system results in feelings of reward and pleasure [12-16], reduced activity of this system (hypodopaminergic functioning) can trigger drug-seeking behavior [17-21].

Hypodopaminergic functioning including reduced DA receptor density, blunted response to DA, or enhanced DA catabolism in the reward pathway, which can be induced by variant alleles or defined polymorphisms have been identified over at least two decades [22]. Cessations of chronic drug use also can produce a hypodopaminergic state that prompts drug-seeking behaviors in an attempt to address the unwanted withdrawal-induced state [23].

1.2. Neurotransmitter mechanisms

Well-being can be produced by acute use of psychoactive substances, however, sustained and prolonged abuse results in tolerance and discomfort [24]. Opioid desensitization/tolerance mechanisms have focused on adaptations that include receptor phosphorylation, internalization, and sub-cellular trafficking on the level of the mu-opioid receptor (MOR). Recent research has revealed augmented isoform-specific synthesis of adenylyl cyclase and their phosphorylation and augmented phosphorylation of the G(beta) subunit of G(beta gamma). These changes result in a shift of mu-opioid receptor-coupled signaling to inhibitory (G(i)-derived) G(beta gamma) stimulatory adenylyl cyclase signaling, from predominantly G(i alpha) [25]. It is noteworthy, that polymorphisms related to MOR have been associated with excessive drug (ethanol) seeking behavior that interacts with dopaminergic pathways in the NAc [26].

A PUBMED (10-24-12) search revealed at least 197 articles dedicated to the role of Dopamine D2 receptor gene and excessive cravings caused by carrying the DRD2 A1 allelic genotype. While a deficit in DA receptors, is compounded by consequential drug seeking behavior, conversely, normal densities of DA receptors result in reduced craving behaviors [18].

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Attenuation of craving to prevent or treat Substance Use Disorder (SUD) could result from proliferation of DA D2 receptors in genetically predisposed individuals [27- 29] and those with hypodopaminergic function, secondary to stress or the toxic effects of the abused substances [30] would also benefit from proliferation of DA D2 receptors.. Boundy et al. [27, 30] have shown, in-vitro, that constant stimulation of the DA D2 receptor system with low doses of a D2 agonist results in significant proliferation of D2 receptors, in spite of genetic antecedents [31]. Proliferation of D2 receptors caused by messenger RNA expression is induced by negative feedback mechanisms in the mesolimbic system, signaled by moderate chronic D2 receptor stimulation [27, 30]. Thus, stimulating rather than blocking dopaminergic receptor sites may be a worthwhile solution to the hypodopaminergic state or trait [32-37]. In nonhuman animals DNA-directed overexpression of the DRD2 receptors induces a significant reduction, in both alcohol and cocaine craving and drug seeking [34-36].

Most recently our laboratory embarked on an unpublished scientific investigation using GARS to assess clients attending two treatment facilities in the United Sates: Malibu Beach Recovery Center, Malibu Beach, California and G&G Holistic Addiction Treatment Center, North Miami Beach, Florida. It is noteworthy that subsequent to the development of an algorithm based stratification of risk assessment of 70 tested patients 100% carried at least one risk allele for RDS behaviors; 5% carried high risk; 81% moderate risk and 14% low risk.

[II] METHODS

Utilizing this genetic test we describe herein one case of a recovering addict's (17 years sobriety) life-history especially as it relates to RDS behaviors and her GARS results. The exercise was to blindly predict clinically relevant information about the person's past behavioral history by identifying individual polymorphic risk alleles **[Table–2]**.The behaviors that associate with them were recorded and blindly compared to the clinical history (Case Study) provided later by the subject.

Table: 2. shows the resultant analysis on EW's (AKA) GARS and individual genotypes

Genes/ Alleles	Results
Caspi MAOA uVNTR	3R
Caspi MAOA uVNTR	4R
DRD4	2R
DRD4	4R
DAT	10R
DAT	10R
5HTTLLR dialletic	S/S
COMT	A/G
DRD2 Taq1	A2/A2
DRD3 C=Gly	C/T
OPRM1 A=Asn G=Asp	G/G
GABRA3	181
GABRA3	197
Allele #	10
Score	0.56
Severity	Moderate

DNA extraction was obtained by saliva collection and genotyped at the Colorado University Institute of Behavioral Genetics utilizing standard techniques [38].

[III] RESULTS

EW has 10 alleles out of the 9 genes with a GARS score of 0.56 which is rather high but fits within the modest Risk. There are 18 alleles for females and 17 alleles for males. Interestingly, EW is not positive for the DRD2 A1, this could have helped her recovery process, whereby the DRD2 A1 has been associated with relapse (Dahlgren et al., 2011) [39]. However, she is positive for MOA gene but is heterozygote 3R/4R, which may result because of the 3R, in a slower breakdown of mitochondrial DA when it is brought back into the presynaptic neuron. Interestingly, EW is polymorphic for the dopamine transporter gene having 10R/10R. This suggests that she may have impulsive tendencies and hyperactivity and possibly ADHD. One noteworthy finding is that EW possesses S/S for the serotonin transporter gene which has been linked to excessive alcohol intake. In terms of the enzyme COMT which breaks down Dopamine in the synapse she carries the AG genotype. The G allele called VAL has been associated with opiate abuse. However, it is clear that she also carries the homozygote of the mu opiate receptor MOR identified as G/G which has been found to endorse drinking to enhance positive affect (liking). She also carries the C/T genotype for the Dopamine D3 gene which has been associated with substance abuse. EW also carries the heterozygote 183 allele of GABA receptor subunit and as such also may like alcohol to relieve her anxiety due to low GABA receptors sensitivity.

[IV] CASE STUDY

EW (AKA) is a 54 year old Caucasian female with a long standing history of polysubstance abuse. Her first use was alcohol at age 13. Over the next few years, she progressed to regular use of benzodiazepines, prescription stimulants, and LSD. At age 19, she began using heroin and cocaine intravenously and quickly became addicted. During this period, she also drank intermittently. She would become violently ill every time she used any opiates or alcohol, but continued to use to modify her feelings. Over the next several years, EW "detoxed" multiple times on methadone, but repeatedly returned to drug use. At the age of 27, she began attending AA. She was able to stay sober for the majority of that time, with three very brief relapses on alcohol, methamphetamine, and benzodiazepines. She currently has 17 years of uninterrupted sobriety.

In sobriety, EW was diagnosed with ADHD and has had constant problems with impulse control. She believes that this played a large part in her relapse history; as well as affecting her personal relationships, social functioning, and overall well-being. EW does have a family history of addiction. Her father, deceased, was a recovering alcoholic. She also reports

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alcoholism in her maternal great-grandfather. Both EW's mother and grandmother used prescription opiates and sedatives to excess. There is a family history of depression and suicide.

[V] DISCUSSION

Homo sapiens in evolutionary terms are changing very slowly, and certain genetic traits such as genes that regulate pleasure seeking may be the exception [32, 33]. Interestingly, the DNA analysis of the discovered Iceman (Ötzi), for the most part, with the exception of the genes responsible for lactose intolerance, atherosclerosis, and having Borrelia burgdorferi making him the earliest known human with Lyme disease, matches to some extent modern day humans. His autosomal DNA is most closely related to southern Europeans, from geographically isolated populations in Sardinia and Corsica but he seems to be closer to Neanderthal ancestry [40] However, we do not know whether the DRD2 A1 allele is an older gene allele or if it is newer than the DRD2 A2 allele. Identifying this will help clarify the nature of the relationship humans have with pleasure-seeking and perhaps how it benefits our survival. For example, carriers of the DRD2 A1 allele are more aggressive than carriers of the DRD2 A2 allele [41-43].

The work of Blum et al. [4] and others including brain imaging studies [44] have helped us explain molecular mechanisms of addiction. One component of all this serious investigation suggests that hypodopaminergic function stimulates cravings, which in turn affects attention to goals. Maintenance of cognitive control is required to override compulsions to use drugs. Cognitive control involves the ability to generate action plans and then monitor actions/behaviors to attain goals [45]. The steady influx of DA that occurs with drug abuse becomes the sole focus of attention. The central goal, is obtaining more drugs. Motivated by cravings for drugs, even though the drugs have long stopped providing pleasure, victims of SUDs and process addictions are caught in a spiral of physical brain changes and the psychological consequences of those changes that lead to further physical and psychological changes and consequences [46, 47].

DA is a key genetically induced deficient neurotransmitter causing in abnormal craving behavior and excessive pleasure seeking. Finding ways to increase DA D2 density, instead of blocking dopaminergic function, may be the best strategy to unlock the elusive addiction riddle and attenuate abuse [32, 46, 48].

[VI] CONCLUSION

New treatment and genetic diagnostic approaches are required in view of our most recent unpublished work derived from studies with CARD.™ Specifically, studies from our laboratory in both in-patient and outpatient facilities utilizing the Comprehensive Analysis of Reported Drugs (CARD)™ found a significant lack of compliance to prescribed treatment medications and a lack of abstinence from drugs of abuse during active recovery [49].

We are proposing a paradigm shift a solution for RDS that embraces the coupling of (1) genotyping of individuals for candidate reward genes to determine stratification of genetic risk for all RDS behaviors (GARS)™ [48,50], (2) the use of slow acting D2 agonist therapy (e.g. KB220Z™) to activate dopaminergic pathways in the NAc (affecting abnormal craving) and other brain regions (affecting decision –making) and (3) the use of CARD™ during active recovery to assess compliance to prescribed treatment medications and abstinence from drugs of abuse.

Potential utilization of these tools may provide the clinician the means to generate better diagnosis and recovery rates. Further research, in terms of reinforcement experiments in nonhuman animal models [51] and human trials, will assist in promotion of these novel strategies for the early diagnosis, prevention, treatment and attenuation of relapse in RDS [52,53] including process addictions [54, 55].

CONFLICT OF INTERESTS

Kenneth Blum, Mary Hauser, B. William Downs, Margaret A. Madigan, and John Giordano have a conflict of interest due to the commercial development of the GARS test co -marketed by LifeGen, Inc and Dominion LLC.

FINANCIAL DISCLOSURE

The work was carried out without any financial support from Dominion Diagnostic. LLC.

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RESEARCH ARTICLE OPEN ACCESS

BIOCHEMICAL VARIATION AS INFLUENCED BY BENZYLAMINOPURINE APPLICATION IN WHEAT GENOTYPES UNDER VARIABLE WATER DEFICIT CONDITIONS

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ABSTRACT

The purpose of this study was to investigate the influence of the addition of different concentrations of benzylaminopurine (BAP) in amelioration of the water deficit in wheat genotypes. BAP concentrations were foliarly applied at vegetative and reproductive stages under tillering and boot leaf stage water stress. Water stress given at the tillering stage and the boot leaf stage had significantly reduced the , hill reaction activity, photosynthetic pigments, starch, proteins and significantly induced the sugars, free amino acids, proline in the leaves of the studied genotypes at both 70 DAS and 100 DAS. Foliar application of BAP @100 µg ml-1 given at the vegetative stage under water deficit conditions had showed the stress ameliorative effect. BAP had reduced the detrimental effects of low water availability through stimulating osmotic adjustment in wheat genotypes. BAP have a positive effect on growth factors during water deficit, as stimulating leaf growth and increasing net photosynthetic rates.

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KEY WORDS

Wheat; water stress; tillers; boot leaf; BAP

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[I] INTRODUCTION

Water deficit and salt stresses are global issues to ensure survival of agricultural crops and sustainable food production [1]. Drought is observed in irrigated areas due to insufficient supply of water and canal closure. Water deficit affects every aspect of plant growth by modifying the anatomy, morphology, physiology, biochemistry and finally the productivity of crop. Early grain development stage is more vulnerable to water stress than vegetative growth. The exogenous application of osmoprotectants, growth promoters and antioxidant compounds to plants has been considered as short term solution to alleviate the adverse effects of stress [2]. When the plant tissues were subjected to drought stress, some physiological and biochemical changes occur. Biochemical attributes such as: free proline content, soluble sugar, total protein, decreased phospholipids in the cell membrane [3] and chlorophyll stability can be used as drought tolerance indicators for selecting drought resistant genotypes [4]. Differences in drought tolerance of wheat cultivar were presented by [5]. It was also reported that high yielded variety affected more under stress condition than low yield one [6].

Phytohormones such as cytokinins (CKs) have been reported to reduce the detrimental effects of low water availability through stimulating osmotic adjustment. The foliar spray of osmoprotectants has gained significant ground during the last decade, because it is a shotgun approach to improve stress tolerance in different crops. Phytohormones such as cytokinins (CKs), have been reported to reduce the detrimental effects of low water availability through stimulating osmotic adjustment [7]. Benzylaminopurine (BAP) is also believed to be ideal for exogenous plant application because it is considerably more stable than natural CKs. It is readily taken up by the plant and not degraded by CK oxidase. Water stress tends to accelerate leaf senescence. CKs tend to reduce senescence by maintaining membrane activity [8] and promoting synthesis and inhibiting degradation of protein. This delay in aging is associated with a maintenance of photosynthetic activity [9], thereby enhancing the plant's ability to recover and regrow following water stress. A synthetic CK, BAP has been reported to have a positive effect on some growth factors during water deficit, as stimulating seed germination, leaf growth [10], and increasing net photosynthetic rates.

[II] MATERIALS AND METHODS

2.1. Experimental site

The present investigation was conducted in experimental area of Department of Botany, Punjab Agricultural University, Ludhiana, during

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Rabi season of 2009 and 2010. Ludhiana, representing the Indo-Gangetic alluvial plains, is situated at 30º-54º`N latitude, 75º-45`E longitude and at a mean height of 247 meters above sea level. It is placed in South-Central plain region of Punjab having subtropical and semi-arid climate.

2.2. Experimental treatments and design

The two genotypes PBW 343 and PBW 527 were raised and treatments were allotted in split plot design. Each treatment was replicated thrice.

2.3. Treatments

T1–Untreated control
T2 –Water-deficit at tillering stage (at 50% level using tensiometer)
T3– Water-deficit at boot leaf stage (at 50% level using tensiometer)
T4 –T2+50 µg ml-1 BAP at vegetative stage
T5 –T2+100 µg ml-1 BAP at vegetative stage
T6 –T2+50 µg ml-1 BAP at vegetative and post-anthesis stage
T7 –T2+100 µg ml-1BAP at vegetative and 50µg ml-1at post-anthesis stage
T8 –T3+50 µg ml-1 BAP at vegetative stage
T9–T3+100 µg ml-1 BAP at vegetative stage

2.4. Crop husbandry

The pre-sowing irrigation (75 mm) was applied, prior to sowing; the soil of the replications was carefully leveled to ensure even distribution of water. All the required field management practices were followed according to the specifications laid out in the "Package of Practices for Rabi crops 2009-2010"a handbook of Punjab Agricultural University, Ludhiana. Recommended dose of fertilizers was applied at the time of sowing. Seeds were sown, after soil become in conditions of sowing, each treatment was allotted rows of four meters length. Inter row and inter plant distance was maintained at 20 cm and 8.5 cm respectively. Weeding and hoeing were carried out manually to keep the crop free from weeds throughout the growth period. Foliar spray of BAP at vegetative stage was given at 60 DAS and at reproductive stage was given at 90 DAS. The observations for the biochemical parameters were recorded after 70 DAS and 100 DAS.

2.5. Data collection

The observations for the biochemical parameters (plant pigments [11], hill reaction activity [12], protein [13], free amino acids [14], proline [15], starch [16], and total soluble sugars [17] were recorded.

2.6. Statistical analysis

For the biochemical estimations the data of genotypes sown under variable water deficits were evaluated and Analysis of Variance (ANOVA) was done.

[III] RESULTS AND DISCUSSION

3.1. Photosynthetic pigments

It was clear from **Supplementary Table–1** that there was an inverse proportional relationship between increasing the severity of drought on one hand and contents of leaves of chlorophyll a, b and total pigments on the other hand. Water stress had significantly decreased the chlorophyll-a content. Water stress at tillering stage decreased the chlorophyll a up to much more extent than that of the water stress given at boot leaf stage. Foliar application of BAP had significantly increased the chlorophyll a content under water stress conditions. From among the genotypes, increase in chlorophyll-a in PBW 527 was more than PBW 343 when it was applied with BAP under the stress conditions. There was overall decrease in chlorophyll b concentration under water deficit conditions. Decreased in the concentration of chlorophyll-b was more in PBW 527 as compared to the PBW 343 under water stress. Foliar application of BAP significantly increased the chlorophyll b under tillering water stress. BAP at its higher concentration was found to be better to increase the chlorophyll-b under water deficit conditions. In the present study water stressed plants showed significant decrease in the total chlorophyll. Foliar application of BAP increased the total chlorophyll under stress conditions.

3.2. Hill reaction activity

The rate of hill activity may be limited by almost all adverse environmental factors. From among the genotypes, PBW 527 had more hill reaction activity than that of the PBW 343 under full turgor conditions. Water stress had significantly decreased the hill activity when it was applied at the stages of the growth of wheat plants i.e. (tillering as well as boot leaf stage). Results pointed out that the decrease was more pronounced when water stress was applied at the tillering stage in PBW 343 and at the boot leaf stage in PBW 527 **[Supplementary Table–2]**. Foliar application of BAP at its higher concentration had significantly increased the hill activity under stress which was given at the (tillering and boot leaf stage) when sprayed at the vegetative stage. But the higher application in addition to the lower application at post anthesis i.e. (T7) was found to be more effective to increase the hill activity under tillering water stress conditions in both the genotypes.

3.3. Proline

Proline is the most common osmolyte accumulated in water stress conditions and the accumulation of these compounds is thought to represent an important adaptive response to drought stress. Proline accumulation is also correlated to the increase in total catabolic amino acids and sugar during stress. The proline level in control plant is also found to vary between varieties of the same crop grown under same physiological, soil and environmental conditions i.e. among the control plants, some cultivars show high values whereas others find low. Results showed that PBW 527 had more proline accumulation than that of the PBW 343 **[Supplementary Table–3]** at 100 DAS. Water deficit had significantly increased the proline content at 70 DAS as well AS 100 DAS. In the present study foliar application of BAP at 60 DAS and 100 DAS had significantly reduced the proline concentration under the stress conditions whether the stress was applied at the tillering stage or the boot leaf stage. BAP had significantly decreased the proline when it was applied at the higher concentration under the tillering water stress as well as under boot leaf water stress at both 70 DAS and 100 DAS in the leaves of studied genotypes.

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3.4. Reducing sugars, Starch, Proteins and Total amino acids

Reducing sugar, proteins and free amino acid values varied significantly among the wheat genotypes. In comparison among the genotypes, PBW 527 possessed high soluble sugar content as compared to the PBW 343 under the control conditions. It was evident that the values of soluble sugars extracted from the leaves in the presently studied genotypes of *Triticum* increased progressively by increasing the deficit period. The increase in sugar content was found to be more drastic when the water stress was given at the boot leaf stage. The increase in level of reducing sugar under water stress may also be ascribed to an increase in starch hydrolysis. Results revealed that foliar application of BAP had significantly increased the sugar content when sprayed at the vegetative stage under tillering water stress as compared to the control. The increase was more pronounced in the PBW 527 **[Supplementary Table–4]**.

Two wheat genotypes chosen on the basis of their different drought tolerance were grown in field and subjected to drought at two stages of development (tillering and the boot leaf stage). Highly significant differences were detected between watered and stressed plants for starch content in both the genotypes, proved that the treatment applied leads to a real water deficit.

The both genotypes had showed the different behavior for starch content values. Results revealed significant variations in the values of starch among the two wheat genotypes under consideration. Drought stress decompose starch and fade it from the plant. It was earlier reported that drought stress causes many changes in the amount of plants carbohydrate and it become clear that with increasing drought stress on leaves, the amount of starch decrease. Water stress had significantly decreased the starch content in flag leaves at 70 DAS and 100 DAS. But the decrease was more recorded under tillering stress as compared to the stress given at the boot leaf water stress.

Results showed that foliar application of BAP had significantly decreased the starch in both the genotypes in the flag leaves at 70 DAS and 100 DAS as compared to the control under the stress conditions whether the stress was given at the tillering stage or at the boot leaf stage.

Results showed that free amino acids were increased at the stage of maturity in PBW 527, but decrease in PBW 343 at the stage of maturity **[Supplementary Table–5]**. Results pointed out that water stress had significantly increased the free amino acid. But the increase was more under boot leaf stress in both the genotypes. The properties of compatible solutes facilitate the maintenance of favorable turgor pressure during water stress and in addition may serve as protective agents by stabilizing proteins. Results showed that foliar application of BAP had significantly increased the free amino acids in both the genotypes as compared to the control under the stress conditions at 70 DAS as well as at 100 DAS.

In leaves of studied genotypes protein content was estimated at two stages of growth and results showed the significant differences between the genotypes under stress treatments. From among the genotypes PBW 527 had more proteins as compared to the PBW 343 at both 70 DAS and 100 DAS. Exposing wheat plants to osmotic stress decreased total protein concentration relative to the control treatments in both the genotypes. The decrease was more pronounced when the water stress was applied at the boot leaf stage. Results showed that foliar application of BAP had significantly increased the protein content in both the genotypes as compared to the control under the stress conditions. Protein content was higher at 70 DAS and lower at 100 DAS in both the genotypes.

[III] DISCUSSION

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers [18]. A reduction in chlorophyll content under drought was found presently. It was also reported that BAP application reduced the reduction in chlorophyll content and maintained it at higher level in the leaves [19]. Reduction of starch was the result of amylase activity that increased soluble sugar [20]. Water stress affects the conversion of sucrose in to starch [21]. Water stress reduced starch content in the shoots of tolerant seedlings as compared to the sensitive ones, but increased sucrose content in the shoots and roots of tolerant seedling, indicating their protective role during stress conditions [22]. This increment was significant at boot leaf stage. Previous data showed that level of these metabolites increased over control at 1.0 mg l-1 CK treatment after which variations were non significant [23]. The decrease in the water availability for transport-associated process leads to change in the concentration of many metabolites followed by distribution in amino acid and carbohydrate metabolism and increase in synthesis of compatible solutes, such as amino acids. Therefore, free amino acid seem to be additional to proline accumulation for deciding tolerance in a given crop species. Compatible solutes are synthesized in response to osmotic stress and can occur at high intracellular concentrations without hindering normal cellular metabolism. Increased protein content as well as depletion of amino acids in the grains of kinetin and ethrel treated plants indicate, efficient incorporation of amino acids into proteins. Kinetin had been reported to maintain higher rate of protein synthesis [24]. Application of BAP on younger and older leaves enhanced the soluble protein content except at few stages [25] are also reported in increasing in protein level under influence of BAP in beans.

[IV] CONCLUSION

From this study it was clearly observed that at the stages of drought and subsequent rehydration compounds with cytokinin (BAP) activities were found the most efficient protectors, enhancing a less pronounced decrease in the intensity of photosynthetic efficiency. BAP is a regulator of leaf senescence and their effects is dramatic particularly when sprayed directly

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on the intact plants. BAP protects the cell membranes and the photosynthetic machinery from oxidative damage by delay of senescence. BAP stimulated chloroplast differentiation and inclusion of BAP induced the formation of greater numbers of chloroplasts in the leaves. Thus BAP promoted development of more leaf area and greater plant survival rates under varied water deficit conditions. A higher amount of amino acids and proteins were observed, accumulation of these metabolites in foliar cells may contribute towards dry mass distribution and osmotic adjustment Thus foliar application of BAP @100 µg ml-1 given at the vegetative stage under water deficit conditions had showed the stress ameliorative effect.

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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SUPPLEMENTARY TABLES (As supplied by authors)

Supplementary Table: 1. Influence of BAP on chlorophyll a, chlorophyll b and total chlorophyll (mg $^{-1}$ gm fresh weight) content in wheat (*Triticum aestivum* L.) genotypes under water stress

GENOTYPE TREATMENTS	CHLOROPHYLL A				CHLOROPHYLL B				TOTAL CHLOROPHYLL			
	PBW343		PBW527		PBW343		PBW527		PBW 343		PBW527	
	70DAS	100DAS	70DAS	100DAS	70 DAS	100DAS	70DAS	100DAS	70DAS	100DAS	70DAS	100DAS
T_1-Untreated control	0.7210	0.6200	0.7307	0.7133	0.2639	0.2212	0.2919	0.2442	0.9849	0.8412	1.0226	0.9575
T_2 –Water-deficit at tillering stage	0.3810	0.5844	0.4203	0.6217	0.2396	0.2168	0.2515	0.2075	0.6206	0.8012	0.6718	0.8292
T_3 Water-deficit at boot leaf stage	0.7216	0.3448	0.7135	0.4222	0.2837	0.1914	0.2630	0.1877	1.0053	0.5362	0.9765	0.6099
CD5%	**0.0767**	**0.0623**	**0.0700**	**0.0657**	**0.0093**	**0.0065**	**0.0098**	**0.0128**	**0.0866**	**0.0688**	**0.0798**	**0.0785**
T_4-T_2+50 µg ml-1BAP at vegetative stage	0.4646	0.5864	0.5073	0.6436	0.2613	0.2168	0.2575	0.2088	0.7259	0.8032	0.7648	0.8524
T_5-T_2+100 µg ml-1BAP at vegetative stage	0.5891	0.5879	0.5363	0.6476	0.2773	0.2074	0.2632	0.2119	0.8664	0.7953	0.7995	0.8595
T_6-T_2+50 µg ml-1 BAP at vegetative and post-anthesis stage	0.3933	0.6059	0.5086	0.6514	0.2618	0.2033	0.2612	0.2150	0.6551	0.8092	0.7698	0.8664
T_7–T_2+100 µg ml-1BAP at vegetative and 50 µg ml-1at post-anthesis stage	0.4652	0.6077	0.5360	0.6535	0.2739	0.2204	0.2681	0.2160	0.7391	0.8281	0.8041	0.8695
CD5%	**0.0618**	**0.0501**	**0.0563**	**0.0529**	**0.0072**	**0.0052**	**0.0077**	**0.0049**	**0.0697**	**0.0553**	**0.0643**	**0.0578**
T_8-T_3+50 µg ml-1BAP at vegetative stage	0.7859	0.4422	0.7409	0.4230	0.3085	0.1960	0.2658	0.1947	1.0944	0.6382	1.0067	0.6177
T_9-T_3+100 µg ml-1BAP at vegetative stage	0.7868	0.4518	0.7546	0.4269	0.3255	0.1963	0.2813	0.1946	1.1123	0.6481	1.0359	0.6215
CD5%	**0.0110**	**0.0151**	**0.0195**	**0.0139**	**0.0094**	**0.0087**	**0.0128**	**0.0128**	**0.0204**	**0.0238**	**0.0323**	**0.0267**

Supplementary Table: 2. Influence of BAP application on Hill reaction activity (mg chlorophyll^{-1} hr^{-1}) in wheat (*Triticum aestivum* L.) genotypes under water stress

Genotype	PBW 343		PBW 527	
Treatments	**70 DAS**	**100DAS**	**70 DAS**	**100 DAS**
T_1-Untreated control	0.2573	0.2292	0.3080	0.3148
T_2 –Water-deficit at tillering stage	0.1111	0.2158	0.1577	0.2630
T_3 Water-deficit at boot leaf stage	0.2573	0.1482	0.2929	0.1150
CD5%	**0.0329**	**0.0182**	**0.0339**	**0.0449**
T_4-T_2+50 µg ml-1 BAP at vegetative stage	0.1369	0.2187	0.1685	0.2590
T_5-T_2+100 µg ml-1 BAP at vegetative stage	0.1568	0.2225	0.1804	0.2794
T_6 -T_2+50 µg ml-1 BAP at vegetative and post-anthesis stage	0.1340	0.2210	0.1688	0.2825
T_7 –T_2+100 µg ml-1 BAP at vegetative and 50µgml-1at post-anthesis stage	0.1581	0.2258	0.1834	0.3073
CD5%	**0.0136**	**0.0137**	**0.0174**	**0.0553**
T_8-T_3+50 µg ml-1 BAP at vegetative stage	0.2593	0.1746	0.3039	0.1386
T_9-T_3+100µg ml-1 BAP at vegetative stage	0.2631	0.2030	0.3176	0.1595
CD5%	**0.0493**	**0.0858**	**0.0256**	**0.0381**

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Supplementary Table: 3. **Influence of BAP application on Free Proline (mg $^{-1}$ gm fresh weight) in wheat (*Triticum aestivum* L.) genotypes under water stress**

Genotype	PBW 343		PBW 527	
Treatments	**70 DAS**	**100 DAS**	**70 DAS**	**100 DAS**
T_1-Untreated control	2.554	3.083	2.219	3.356
T_2 –Water-deficit at tillering stage	6.163	3.142	4.050	4.244
T_3 Water-deficit at boot leaf stage	2.780	5.959	2.277	7.371
CD5%	**0.8129**	**0.6481**	**0.4132**	**0.9075**
T_4-T_2+50 µg ml-1BAP at vegetative stage	4.138	3.334	3.988	3.625
T_5-T_2+100 µg ml-1BAP at vegetative stage	3.679	3.212	3.818	3.431
T_6 -T_2+50 µg ml-1 BAP at vegetative and post-anthesis stage	4.009	3.134	2.841	4.187
T_7 –T_2+100 µg ml-1BAP at vegetative and 50µgml-1at post-anthesis stage	3.515	3.062	2.659	3.916
CD5%	**0.6544**	**0.0603**	**0.3326**	**0.6319**
T_8-T_3+50 µg ml-1BAP at vegetative stage	2.454	4.959	2.185	4.953
T_9-T_3+100µg ml-1BAP at vegetative stage	2.096	4.208	2.118	4.271
CD5%	**0.0335**	**0.6469**	**0.0420**	**0.9055**

Supplementary Table: 4. **Influence of BAP application on Sugar and starch (mg $^{-1}$ gm fresh weight) in wheat (*Triticum aestivum* L.) genotypes under water stress**

GENOTYPE TREATMENTS	SUGARS				STARCH			
	PBW343		PBW527		PBW343		PBW527	
	70 DAS	100DAS	70DAS	100DAS	70 DAS	100DAS	70DAS	100DAS
T_1-Untreated control	11.044	17.416	14.245	18.194	5.695	4.053	6.871	4.551
T_2 –Water-deficit at tillering stage	13.017	18.088	17.773	19.032	5.059	3.333	6.530	3.931
T_3 Water-deficit at boot leaf stage	11.424	22.052	15.011	19.716	5.563	3.603	6.904	4.111
CD5%	**0.4449**	**1.203**	**1.474**	**0.3433**	**0.1438**	**0.1423**	**0.1400**	**0.0844**
T_4-T_2+50 µg ml-1BAP at vegetative stage	13.376	18.801	17.917	20.012	4.657	3.153	5.680	3.351
T_5-T_2+100 µg ml-1BAP at vegetative stage	13.444	18.934	18.581	20.068	4.741	2.863	5.801	3.341
T_6-T_2+50 µg ml-1 BAP at vegetative and post-anthesis stage	13.378	21.128	18.496	19.869	4.693	2.953	5.724	3.661
T_7–T_2+100 µg ml-1BAP at vegetative and 50µgml-1at post-anthesis stage	13.457	21.266	18.620	20.032	4.717	2.813	5.856	3.621
CD5%	**0.0682**	**0.9689**	**0.8736**	**0.2765**	**0.1155**	**0.1145**	**0.1123**	**0.0679**
T_8-T_3+50 µg ml-1BAP at vegetative stage	11.476	22.940	15.036	20.612	5.017	2.843	6.175	3.781
T_9-T_3+100 µg ml-1BAP at vegetative stage	11.976	23.332	15.046	20.640	5.065	2.783	6.351	3.511
CD5%	**0.0444**	**0.0773**	**0.0023**	**0.3434**	**0.1435**	**0.1423**	**0.1403**	**0.0846**

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Supplementary Table: 5. **Influence of BAP application on total amino acids and Protein content (mg $^{-1}$ gm fresh weight) in wheat (*Triticum aestivum* L.) genotypes under water stress**

GENOTYPE TREATMENTS	FREE AMINO ACIDS				PROTEIN CONTENT			
	PBW343		PBW527		PBW343		PBW527	
	70 DAS	100DAS	70DAS	100DAS	70 DAS	100DAS	70DAS	100DAS
T_1-Untreated control	11.044	17.416	14.245	18.194	6.450	5.639	7.724	5.257
T_2 –Water-deficit at tillering stage	13.017	18.088	17.773	19.032	5.039	5.154	6.527	5.008
T_3 Water-deficit at boot leaf stage	11.424	22.052	15.011	19.716	6.374	4.273	7.752	4.166
CD5%	**0.4449**	**1.203**	**1.474**	**0.3433**	**0.0932**	**0.1097**	**0.0432**	**0.0553**
T_4-T_2+50 µg ml-1BAP at vegetative stage	13.376	18.801	17.917	20.012	6.221	5.219	7.621	5.029
T_5-T_2+100 µg ml-1BAP at vegetative stage	13.444	18.934	18.581	20.068	6.288	5.231	7.677	5.044
T_6-T_2+50 µg ml-1 BAP at vegetative and post-anthesis stage	13.378	21.128	18.496	19.869	6.249	5.355	7.649	5.064
T_7–T_2+100 µg ml-1BAP at vegetative and 50µgml-1at post-anthesis stage	13.457	21.266	18.620	20.032	6.268	5.426	7.687	5.084
CD5%	**0.0682**	**0.9689**	**0.8736**	**0.2765**	**0.0753**	**0.0879**	**0.0356**	**0.0441**
T_8-T_3+50 µg ml-1BAP at vegetative stage	11.476	22.940	15.036	20.612	6.546	5.438	7.949	5.217
T_9-T_3+100 µg ml-1BAP at vegetative stage	11.976	23.332	15.046	20.640	6.613	5.456	8.099	5.247
CD5%	**0.0444**	**0.0773**	**0.0023**	**0.3434**	**0.0929**	**0.0022**	**0.0458**	**0.0006**

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RESEARCH ARTICLE OPEN ACCESS

SIMPLE SEQUENCE REPEATS IN SPECIFIC GENE GROUPS OF SHIGELLA GENOME

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ABSTRACT

In this paper we attempt to analyze the phenomenon of simple sequence repeats (SSRs) variations in Clusters of Orthologous Groups of proteins (COGs) and horizontal transfer genes (HGT) of Shigella flexneri. We have performed a detailed comparative study of the distribution of SSRs in different gene clusters. According to our finding SSR elements in Shigella pathogenicity islands (PAIs) are significantly overrepresented than in other gene clusters of Shigella particularly in sfII islands which have implications in Shigella virulence and also in virulence genes of 2 Shigella plasmids. The trinucleotide groups of SSRs, the codon repetitions and the amino acid repeats have biased distribution in different gene clusters. Data have been found in this study are subject to; (I) Strong selection of SSRs in PAIs which have important roles in Shigella virulence and (II) important roles of SSRs in determining protein function and genetic development.

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KEY WORDS

SSR; Shigella; gene clusters; PAI; COGs

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[I] INTRODUCTION

Simple sequence repeats (SSRs) may provide an evolutionary advantage; they may function as evolutionary tuning knobs by allowing fast adaptation to new environments [1, 2]. Numerous lines of evidence have demonstrated that genomic distribution of simple sequence repeats (SSRs) is nonrandom, presumably because of their effects on chromatin organization, regulation of gene activity, recombination, DNA replication, cell cycle, mismatch repair (MMR) system, etc [3]. Recently, however, many reports have demonstrated that a large number of SSRs are located in transcribed regions of genomes, including protein-coding genes and expressed sequence tags (ESTs) [4], although in general, repeat numbers and total lengths of SSRs in these regions are small [5,6]. Debates over whether SSRs play any functional role in organism development, adaptation, survival, and evolution are never-ending. The currently available information on the location of specific SSRs in known genes and ESTs permits the unraveling of the biological significance of SSR distribution, expansion, and contraction in the functioning of the genes themselves [7].

Prokaryotic and eukaryotic repeat families are clustered to non homologous proteins. This may indicate that repeated sequences emerged after these two kingdoms had split. The eukaryotes incorporating more repeats may have an evolutionary advantage of faster adaptation to new environments [8]. In a variety of organisms, it has been demonstrated that microsatellite mutation rates are positively correlated with repeat number [9]. In prokaryotes, strong positive selective pressures are associated with highly mutable microsatellite tracts that control pathogenicity [10].

The presence of SSRs in prokaryotes is rare, but most that do occur are related to pathogenic organisms; their variation in repeat numbers can also cause phenotypic changes [11]. Haemophilus influenzae (Hi), an obligate upper respiratory tract commensal/pathogen, uses phase variation (PV) to adapt to host environment changes. Switching occurs by slippage of SSR repeats within genes coding for virulence molecules. When SSR repeats lie within protein coding regions, UTRs, and introns, any changes by replication slippage and other mutational mechanisms may lead to changes in protein function [12].

Shigella is an important human pathogen, responsible for the majority of cases of endemic bacillary dysentery prevalent in developing nations [13]. Shigellosis is common among children less than five years of age in developing countries and in persons who travel from industrialized to less developed countries [14].

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In this paper, we attempt to analyze the phenomenon of SSR variation in clusters of orthologous groups of proteins (COGs) and horizontal transfer genes (HGT) of *Shigella flexneri* as it is common among children in developing countries. We have performed a detailed comparative study of the distribution of SSRs in different gene clusters.

[II] MATERIALS AND METHODS

2.1. DNA sequences

Genome sequences were obtained from ftp://ncbi.nlm.nih.gov/genbank/genomes/.
Gene groups Distributed by COGs (Clusters of Orthologous Groups of proteins) were obtained from http://www.ncbi.nlm.nih.gov/sutils/coxik.cgi?gi=257.
Pathogenicity islands of *Shigella flexneri* 2a str 301 were determined using http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=NC_004337.
Pseudogenes, plasmid virulence genes, and chromosome virulence genes were determined by using SHIBASE, an integrated database for comparative genomics of *Shigella*, at http://www.mgc.ac.cn/ShiBASE/VFs.htm

2.2. Analysis methods

We used the software developed by Gur-Arie et al [15] to screen the COGs, *Shigella* islands, pseudogenes, and virulence genes of *Shigella* for SSRs with a motif length between 1 and 10 bp and a minimal number of three repeats and an entire SSR array length of at least two. This software can be downloaded from ftp://ftp.technion.ac.il/supported/biotech/ssr.exe.

Filter of DNA is done by using http://puma.icb.usp.br/sms/filter_dna.html, for remove non-DNA characters from text including digits and blank spaces from a sequence.
Shuffle nucleic acid sequence has done to produce a randomized sequence with the same overall composition as the original sequence by useing sequence shuffling tool downloaded from http://bcf.arl.arizona.edu/resources/online_tools/shuffle.php.

2.3. Calculation of the expected number of mononucleotide SSRs

To compare the observed number of SSRs with the expected number, we calculated the expected number of homo-oligomer tracts of t bases in a sequence of length N using the formula given by De Wachter T1,t = ∑ P ti (1-Pi)×[(N-t-1)(1-Pi)+2] (summed over i = 1 – 4), with pi being the frequency of each base in the sequence (R).

[III] RESULTS

The Using above mentioned computer programs, we analyzed most important gene groups/mechanisms of *Shigella* which have a important role in *Shigella* function and pathogenicity. The frequency of total SSRs (%5.49) and mononucleotide SSRs (%5.28), with a minimal repeat of 3 in *Shigella* islands, is higher than the other clusters. The frequency of dinucleotide SSRs in *Shigella* islands and transcription genes (%0.17) as well as trinucleotide SSRs in translation genes (%0.06) are higher, followed by intracellular trafficking and secretion genes (%0.053). The frequency of total SSRs (%4.23) and mononucleotide SSRs (%4.18) is lower in transport genes **[Table–1]**. The ratio of observed mononucleotide SSRs to expected mononucleotide SSRs for A and T is greater than 1 and for C and G smaller than 1, except for IS elements and A8 in cell wall/membrane genes and T8 in translation genes. The ratios of observed mononucleotide SSRs to expected mononucleotide SSRs for A3–A5 and T3–T5 for different gene clusters are nearly identical but increases with increasing motif length differences. The ratio of A and T mononucleotide SSRs with increasing motif length was shown to increase, except for A8 and T8, which decrease. The ratios of A6–A8 of *Shigella* islands are much higher than those of other gene groups. The ratio of observed mononucleotide SSRs to expected mononucleotide SSRs in IS elements is totally different from other gene groups **[Figure–1]**. Genes with a high GC content have a lower mononucleotide and dinucleotide SSR density than genes with a low GC content. The numbers of mononucleotide and dinucleotide SSRs are negatively correlated with GC content (Figure 2**)**. The A/T compositions of mononucleotide repeats in all investigated genes are much higher than the C/G compositions of those repeats. Differences were significant by X^2 test ($p < 0.0001$) **[Figure–3]**. In dinucleotide SSRs the frequency of GC/CG in all investigated genes except *Shigella* islands, are higher while frequency of AT/TA in most of genes are lower. Most difference between frequency of CG/GC and AT/TA has observed in the amino acid transport genes (%62.9 and %4.3 respectively) and least difference has observed in the *Shigella* islands (%23.8 and %15.0 respectively). The frequency of AC/GT dinucleotide repeats is higher in intracellular trafficking and secretion genes and *Shigella* islands than other genes. The frequency of AG/CT dinucleotide repeats is higher in posttranslational modification, protein turnover, chaperones genes and signal transduction mechanisms genes. The frequency of TC/GA dinucleotide repeats is higher in signal transduction mechanisms genes and repair, recombination, replication genes. The frequency of TG/CA dinucleotide repeats is higher in repair, recombination, replication genes and *Shigella* islands **[Figure–4]**.

The codon repetition of CAG for (Gln) and GCG for (Ala) are most abundant in all gene clusters except translation genes. In translation genes ACG for (Thr) and CGC for (Arg) are the most abundant. Alanine and Arginine repetition are strongly overrepresented in most of the gene clusters. Alanine and serine repetition in the signal transduction mechanism genes as well as alanine and glutamine in the replication recombination and repair genes are strongly overrepresented. Arginine and glutamine in the inter trafficking and secretion genes are overrepresented.

In contrast the frequency of amino acid repeats in *Shigella* islands is nearly identical and there is no overrepresentation of alanine or arginine such as other gene groups. Differences between frequency of amino acid repeats in *Shigella* islands

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and other gene groups were significant by $\chi 2$ test ($P<0.001$) [Figure–5].
The frequency of mononucleotide SSRs in Sf II island are higher than other SIs gene groups and it is lower in Sci islands [Table–2]. The percentage of mono, di, tri and total SSRs ≥3bp in mxi-spa genes are more than vir genes in both investigated plasmids [Table–3].

Table: 1. Frequency of SSRs in different gene clusters of *Shigella.f 2a str 301*

Genes	Length bp		Total SSRs		Mono nucleotide Repeats		Di nucleotide Repeats		Tri nucleotide Repeats	
	N	%	N	%	N	%	N	%	N	%
IS	303097	6.6	13447	4.43	13080	4.31	322	0.11	45	0.015
SI	430105	9.3	23620	**5.49**	22731	**5.28**	741	**0.17**	141	0.032
PS	363034	7.9	17330	4.77	16720	4.6	476	0.13	131	0.036
RRR	423084	9.3	18387	4.34	17587	4.16	446	0.11	93	0.022
TrS	135834	3.0	5975	4.4	5699	4.2	197	0.15	75	**0.06**
TrC	192078	4.2	9021	4.7	8621	4.5	322	**0.17**	74	0.04
CWMM	244116	5.4	11843	4.85	11349	4.65	384	0.16	97	0.04
EP	292344	6.3	13151	4.5	12555	4.3	446	0.15	134	0.046
TR	1093803	23.7	46376	**4.23**	44479	**4.18**	1645	0.15	468	0.043
InCTS	95832	2.1	4468	4.66	4405	4.6	107	0.11	51	**0.053**
PTMPTC	113073	2.4	5167	4.57	4812	4.3	136	0.12	58	0.05
STM	137145	3.0	6478	4.73	6232	4.53	191	0.14	53	0.04
tRNA	7475	0.16	326	4.36	317	4.24	8	0.11	1	0.013
rRNA	32821	0.71	1723	5.25	1675	5.1	43	0.13	5	0.015

IS: Insertion Sequences; **PS**: Pseudogenes; **SI**: *Shigella* islands; **RRR**: Repair, recombination, replication; **TrS**: Translation; **TrC**: Transcription; **CWMM**: Cell wall/ Membrane Mechanism; **EP**:Energy production; **TR**: Transport Genes; **InCTS**: Intracellular trafficking and secretion; **PTMPTC**: Posttranslational modification, protein turnover, chaperones; **STM**: Signal transduction mechanisms

Table: 2. SSRs in 4 groups of *Shigella* Islands with sizes >1 kb in chromosome of *Shigella.f 2a str 301*

Mono nucleotide Repeats	Sci islands 21440bp		ipaH islands 98767bp		SHI-1 &2 80483bp		Sf II island 28913bp	
	N	%	N	%	N	%	N	%
Mono	834	**3.89**	4918	4.98	3982	4.95	1482	**5.13**
Di	30	0.14	138	0.14	135	0.17	57	0.2
Tri	8	0.037	35	0.035	30	0.037	8	0.03
Tetra	0	0.0	2	0.002	0	0.0	0	0.0
Total	872	**4.1**	5093	5.16	4147	5.15	1547	**5.35**

Table: 3. Frequency of SSRs in Vir genes and Mxi Spa genes of plasmids pCP301 and pSD1_197

Plasmids	pCP301						pSD1_197					
Virulence genes	Mxi Spa genes 25551bp		Vir genes 32551bp		Total 58102bp		Mxi Spa genes 25448bp		Vir genes 27293bp		Total 52741bp	
SSRs	N	%	N	%	N	%	N	%	N	%	N	%
Mono≥3bp	1571	**6.1**	1781	5.5	3352	5.77	1566	**6.2**	1463	5.36	3029	5.74
Di≥3bp	70	0.27	66	0.2	136	0.23	68	0.3	54	0.2	122	0.23
Tri-Hexa≥3bp	12	0.05	6	0.02	18	0.03	8	0.03	8	0.03	16	0.03
Total≥3bp	1653	**6.47**	1853	5.69	3506	6.03	1642	**6.45**	1525	5.59	3167	6.0

Vir genes including: icsA, ipaA, ipaB, ipaC, ipaD, ipaH, ipah1.4, ipaH4, ipaH7.8, ipaH9.8, ipaJ, ipgA, ipgB1, ipgB2, ipgC, ipgD, ipgE, repA, repB, virA, virB, virK.
Mxi-spa gene including: MxiA, MxiC, MxiD, MxiE, MxiG, MxiH, MxiI, Mxij, MxiK, MxiL, MxiM, MxiN, ospB, ospC1, ospC2, ospC4, ospD1, ospD2, ospD3, spa13, spa15, spa24, spa29, spa32, spa33, spa40, spa47, spa orf10

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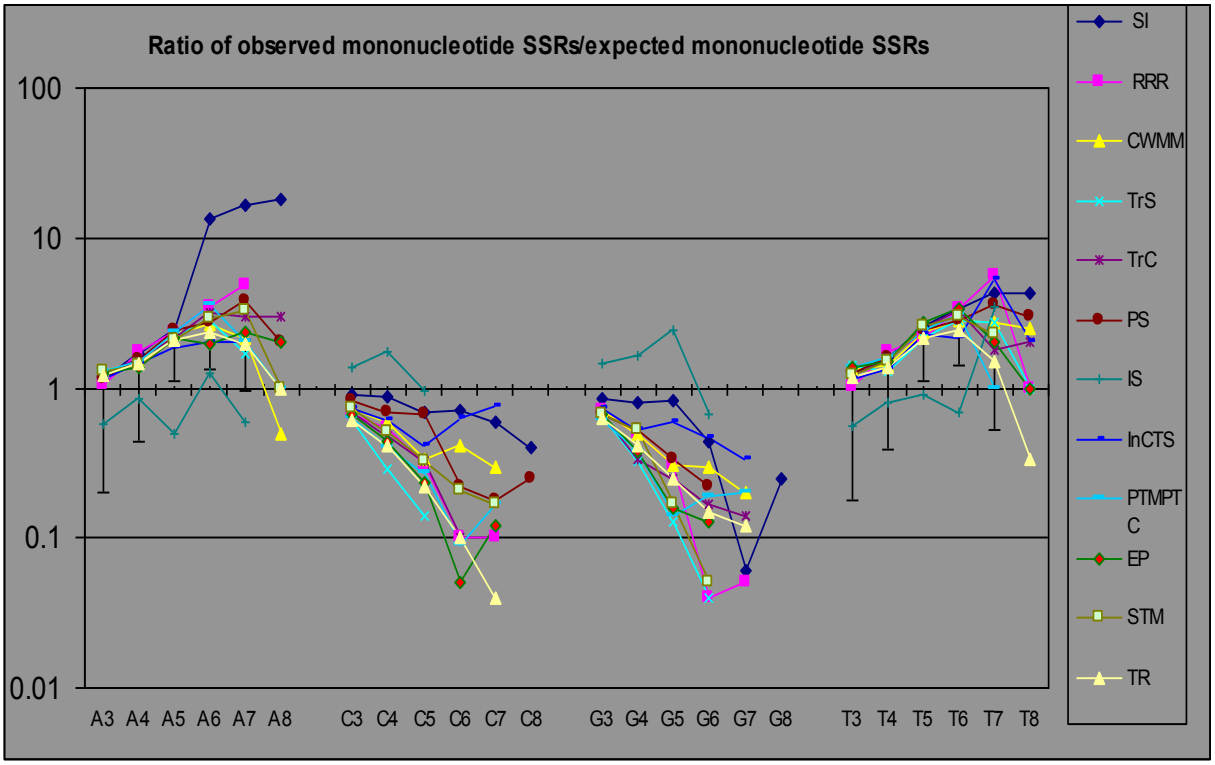

Fig: 1. Ratio of observed Mononucleotide SSRs /expected Mononucleotide SSRs

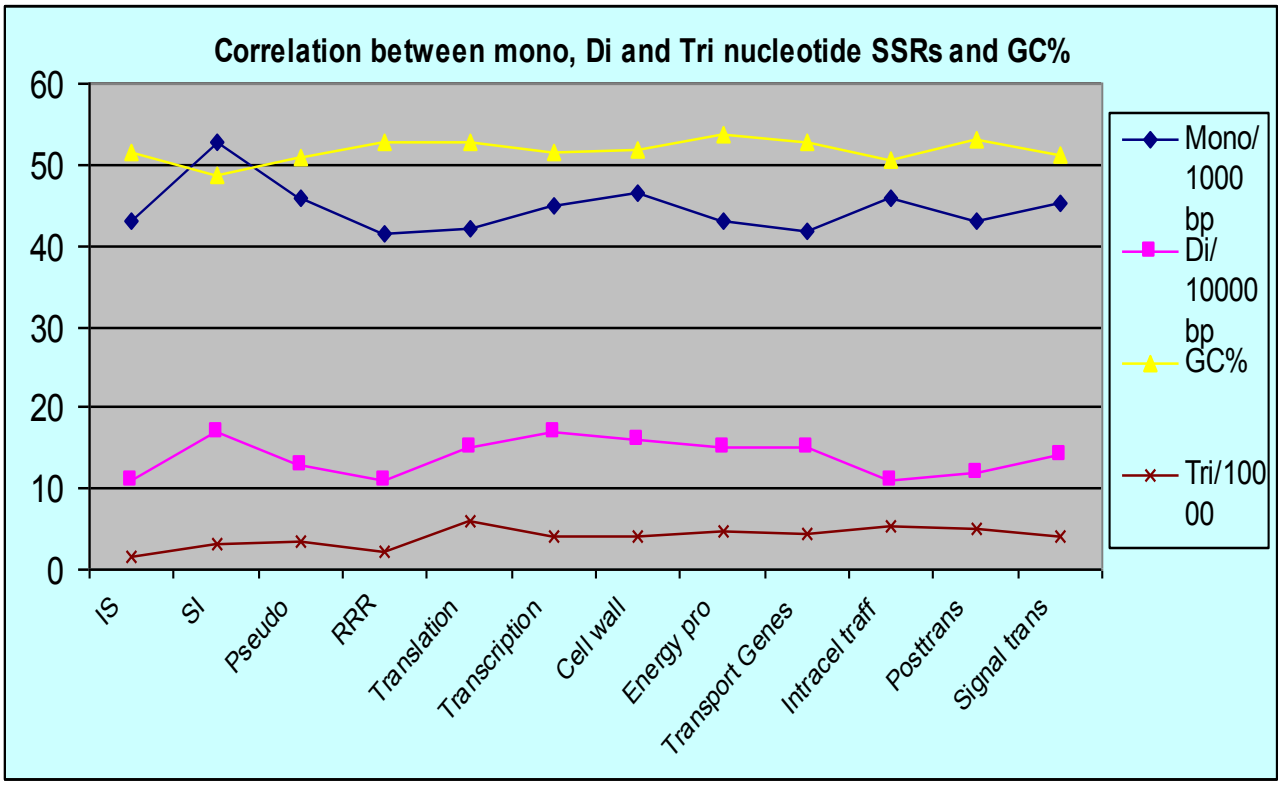

Fig: 2. Correlations between mono, di and trinucleotide SSRs with GC%in different gene clusters

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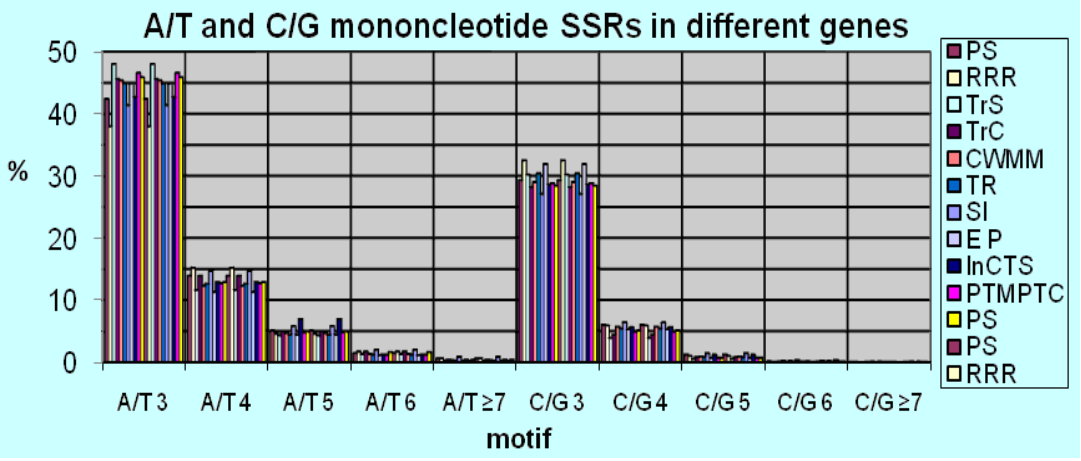

Fig: 3. A/T and G/C mononucleotide SSRs in different gene clusters of *Shigella.f 2a str301*

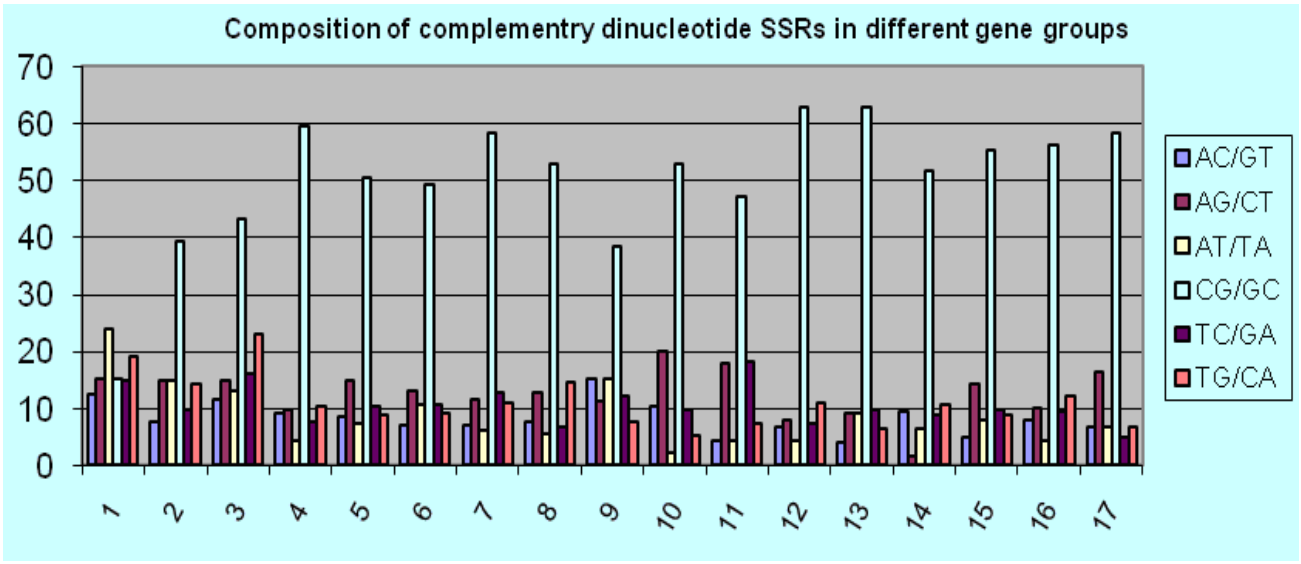

Fig: 4. Composition of complementary dinucleotide SSRs in different gene clusters of *Shigella.f 2a str 301*
1:SI 2:PS 3:RRR 4:TrS 5:TrC 6:CWMM 7:TR 8:EP 9:InCTS 10:PTMPTC 11:STM 12:Amino Acid Transport 13;Coenzime Transport 14:Carbohydrate Transport 15:Inorganic Ion T 16;Lipid Transport 17:Nucleotide Transport

[IV] DISCUSSION

4.1. Association of SSRs with protein function

Although in prokaryotes SSRs are not as abundant as in eukaryotes, most of the SSRs in bacteria are located in virulence genes and/or regulatory regions, and they affect pathogenesis and bacterial adaptive behavior, indicating the signature of natural selection [12, 16]. This hypothesis has been supported by our finding. For instance in our finding SSR in *Shigella* Pathogenicity islands are overrepresented than in other gene groups of *Shigella*, particularly ipaH islands, sf II island and SHI-1&2 which have implications in *Shigella* virulence. Thus, phage-mediated horizontal DNA transfer appears to be one of the major routes by which *Shigella flexneri* gains virulence determinants.

However regarding to overrepresentation of SSRs in ipaH, there is evidence that *S. flexneri* expresses more IpaH within host cells, and the proteins penetrate the host cell nuclei [17]. This, and the fact that all IpaH proteins have a leucine-rich repeat region found in a diverse group of proteins from bacteria and eukaryotes [18], implies that IpaH might be involved in manipulating host gene expression.

Biased distribution of Codon repetition and amino acid repeats

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have been found in different gene groups, suggesting that repeats of these kinds are subject to strong selection. Functional associations of amino acid repeats for such a scenario to be valid, amino acid repeats of this kind must be associated in some way with protein function.

Our data has shown the overrepresentation of SSRs in ipaH genes and mxi- spa genes of Plasmids pCP301 and pSD1_197. While the Ipa proteins are essential for the invasion of epithelial cells, and their secretion is mediated by the proteins encoded at the mxi and spa loci [19, 20], the SSRs overrepresentation indicates opportunity of adaptability under different system environment.

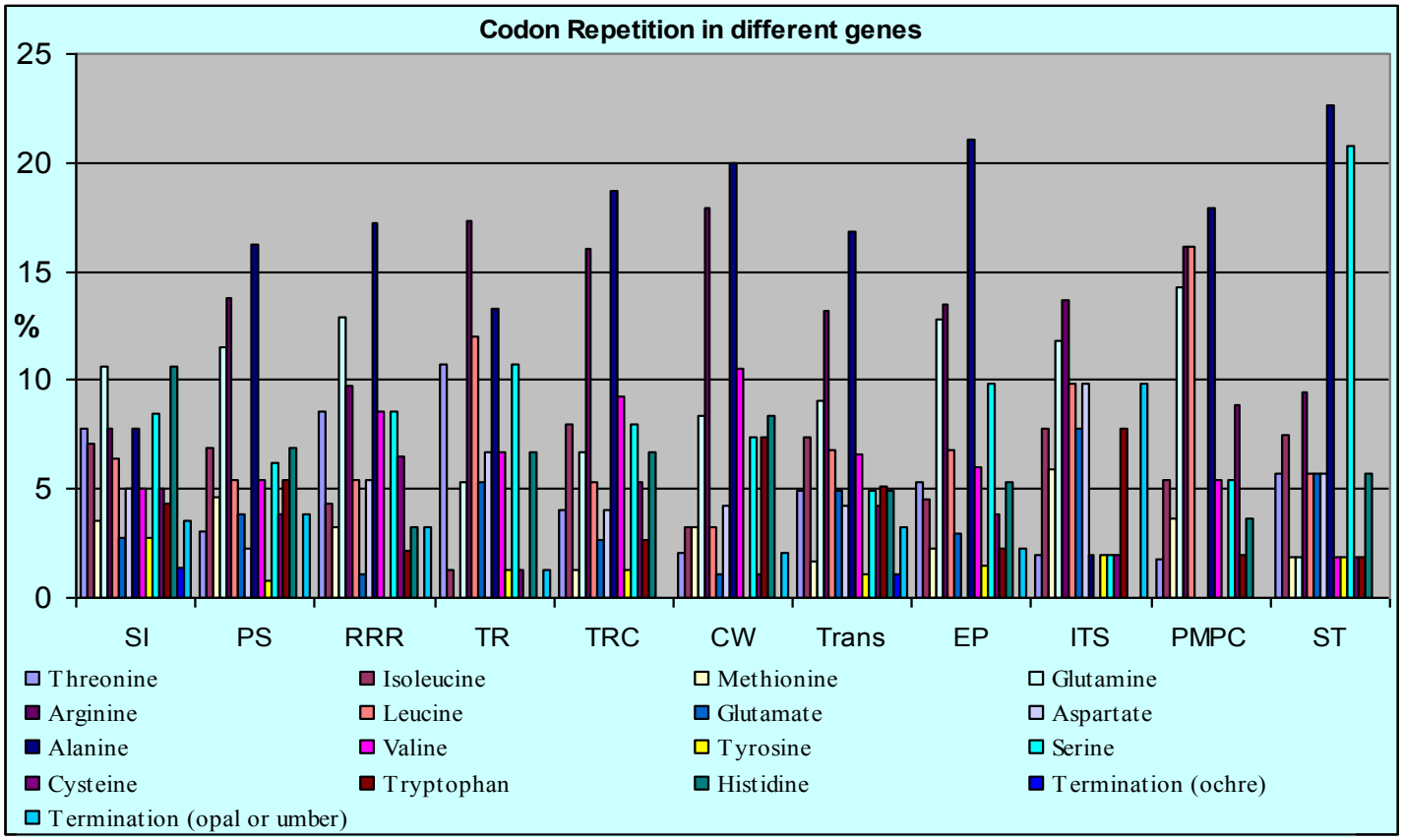

Fig: 5. Frequency of Codon repetition in different gene groups of *Shigella.f 2a str 301*

4.2. SSRs in Shigella Pathogenicity islands >1 kb

The Overrepresentation of total SSRs (%5.49) and mononucleotide SSRs (%5.28) in *Shigella* islands compare with other gene groups (%4.47 and %4.28 respectively), more occurrence of SSRs with increasing size of motif length in *Shigella* islands compare to other gene groups, differences on amino acid repeats pattern and codon repeats frequency between *Shigella* islands and other gene groups, high frequency of AT/TA dinucleotide and low frequency of GC/CG dinucleotide SSRs than the other gene clusters, shows differences between genetic structure of *Shigella* islands (horizontal genes transfer) and other COG genes. These differences may be associated with their evolution or may be generated after integration of PAI-specific DNA regions into the host genome via recombination [21]. It has been speculated that changes in length of repeats in such systems could alter their behavior and therefore contribute to their evolutionary diversification [22, 23], perhaps involving molecular co evolution between proteins [23].

Strongly biased distributions of the all SSR elements in *Shigella* islands have been found in this study emphasize the importance of SSRs in these PAIs which have important roles in *Shigella* virulence.

4.3. SSRs in specific gene groups located in large Shigella Islands>1 kb

Our investigation of SSRs in Sci islands, ipaH islands, SHI-1 and 2 islands and sf II islands, and their implications on the role of virulence, clearly shows differences between frequencies of total and mononucleotide SSRs and the composition of mononucleotide and dinucleotide SSRs between them, particularly between Sci islands and sf II island. This may be

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associated with their evolution because these islands share homology with genes of different phages. For example, chromosomal ipaH islands are originally linked with phage P27. The Sci island possesses a typical structure of PAI–inserted at an asp-tRNA, and ends with an IS629 on the other side. It also carries paralogs of the Salmonella sci CDEFF operon of unknown function and of phages P22 and HK620. The Sf II island has been demonstrated to be a lysogenic phage required for the expression of the type II antigen. It appears to be one of the major routes by which *S. flexneri* gains virulence determinants. Significant differences occur between SSR elements in the Sf II island with other SIs gene groups by X2 test (P=0.01), including overrepresentation of mononucleotide SSRs, a higher frequency of A/T mononucleotide SSRs and higher frequency of AT/TA dinucleotide SSRs. This may be associated with gene function. By analyzing the Sf II island we found that this island involves 37 proteins, including 12 hypothetical proteins (%5.1 SSR), 12 IS elements (%5.6 SSR), four phage integrases (%6.0 SSR), two putative glucosyl tranferases (%6.9 SSR), and seven other genes (%4.5 SSR). Our investigation has shown that SSR in putative glucosyl tranferases and phage integrases is higher than the other genes, implying the involvement of these genes in pathogenic activities.

4.4. Correlation of GC content and SSRs

Our data clearly indicate that the GC content of mononucleotide SSRs is highest when the repeat density is lowest. This suggests that there could be other reasons for the tremendous overrepresentation of poly (A) and poly (T) mononucleotide SSRs. It has been suggested that the higher energy cost of G and C over A and T/U could be the reason for the high variation seen in genomic G+C content. Indeed, the synthesis of GTP requires an additional NAD compared with AMP, while the synthesis of CTP from UTP requires an additional ATP molecule. In addition, due to its central role in metabolism, ATP is abundantly present in the cell [24, 25].

[V] CONCLUSION

In conclusion, the present study has shown biased distribution of trinucleotide groups of SSRs, Codon repetition and amino acid repeats in different gene clusters in *Shigella*. Significant differences between SSR patterns in *Shigella* Pathogenicity islands with other gene groups of *Shigella* are also manifested. The overrepresentation of SSRs in ipaH genes and Mxi, Spa gene particularly plasmid ipaH genes are correlated with pathogenicity of *Shigella*.

Strongly biased distributions of the all SSR elements in *Shigella* islands have been found in this study, emphasize the importance of SSRs in these PAIs which have important roles in *Shigella* virulence.

Study has suggested that SSRs in different positions of a gene can play important roles in determining protein function, genetic development, and regulation of gene expression.

CONFLICT OF INTERESTS

Authors declare no conflict of interests

FINANCIAL DISCLOSURE

The work was carried out without any financial support

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COMMENTARY OPEN ACCESS

THERAPEUTIC POTENTIAL OF LET-7, MIR-125, MIR-205, AND MIR-296 IN BREAST CANCER: AN UPDATE

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ABSTRACT

In 2008, first time we hypothesized that Let-7, miR-125, miR-205, and miR-296 may be potential next-generation therapeutics in breast cancer. In recent years, various reports have supported our hypothesis and it seems that in near future these miRs are highly likely to be used for breast cancer therapy. This commentary summarized the recent findings towards establishing our report of 2008.

Key words: Breast cancer; cancer therapy; miRNA; molecular medicine

COMMENTARY

In 2008 we reported that Let-7, miR-125, miR-205, and miR-296 could be potential therapeutics in breast cancer [1]. In that work we had shown, Let-7 could target estrogen receptor, mitogenic, and angiogenic signaling pathways and thereby blocks cell cycle, cell proliferation, cell migration, angiogenesis, and metastasis in breast cancer. For miR-125, miR-205, and miR-296, we had predicted that, these miRs can precisely inhibit various growth receptor signaling cascades and positive regulators of cell cycle.

The current experimentally validated knowledge of these four miRs in respect to breast cancer supports our hypothesis of 2008 [1]. Let-7 based therapeutic approaches in lung cancer [2] had already been established before our report. However, several validation reports after our publication in 2008 strongly suggest that Let-7 will also be an effective therapeutic in breast cancer. Let-7 is a tumor suppressor miRNA and is downregulated in breast cancer [3]. Whereas, Let-7b inhibits estrogen receptor signaling [4], induces TP53 mediated apoptosis [5]; Let-7a and Let-7d reported to inhibit cell cycle and cell proliferation [6-7]. Chang et al in 2011 have showed that Let-7d prevents epithelial to mesenchymal transition and cell migration [8]. Further, Zhao et al (2011) reported that downregulation of Let-7d makes breast cancer resistance to tamoxifen [4]. The latest report reveals that, Ectopic expression of Let-7b inhibits cell migration in breast cancer [9]. Therefore, administration of Let-7 miRNA might be a future therapeutic in drug registrant and estrogen positive metastatic breast cancers.

Similar to the Let-7; miR-125 is a putative tumor suppressor miRNA and miR-125a-5p is downregulated in ductal breast cancers [10]. Mutation in the miR-125a-5p gene is reported to be associated with hereditary breast cancers [11]. This miRNA inhibits cell proliferation, cell migration, and induces apoptosis also [12-13]. The third miRNA we identified in 2008 was miR-205 [1]. According to Song and Bu (2009) miR-205 inhibits cell migration [14]. miR-205 is downregulated in breast cancer [3] and induced expression inhibits cell proliferation in breast cancer [15]. It also makes the breast cancer cells susceptible to Lapatinib [16]. The last miRNA we reported was miR-296. This miRNA later reported to be involved in regulation of apoptosis [17] and negative regulation of cell migration [18]. Further, Vaira et al (2012) also showed that this miR-296 is downregulated in breast cancer and its ectopic expression inhibits cell proliferation in breast cancer [18].

CONCLUSION

Last four year's (2008-2012) experiments by various research groups suggest that our proposed Let-7, miR-125, miR-205, and miR-296 based therapeutics in breast cancer [1] could be recognized in near future. Let-7 based Lung cancer therapy is already at clinical trial level. The cell cycle, cell proliferation, cell migration, angiogenesis, and metastasis inhibitory effects of these four miRNAs in breast cancer cells have now been established by various researchers after our report in 2008. We hope that, very soon these miRNAs will enter into clinical trial towards establishing them as next-generation breast cancer therapeutics.

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SHROT COMMUNICATION | OPEN ACCESS

EFFECT OF DIFFERENT LIGHTS ON THE SEED GERMINATION OF HIPPOPHAE SALICIFOLIA

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ABSTRACT

Hippophae salicifolia D.Don (Vernacular – Chuk. Tarwa) is a deciduous tree species restricted to the Himalayan region, between 1500-3500 m a.m.s.l. Seeds of H. salicifolia were collected in the month of October 2009 from Uttarakhand State and exposed to different lights. Experiments were conducted in order to investigate germination behavior of H. salicifolia seeds subjected to different lights (red, blue, green, yellow and white as control). The experiments were conducted with four replications in each treatment and twenty five seeds per replication. The results of the study revealed that maximum germination percentage was found under red and yellow lights. Maximum radicle and plumule length was observed in white light (control) but minimum under green light. The study establishes the colour dependence of germination of the seeds of this species.

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KEY WORDS

Hippophae salicifolia, Himalayan, deciduous, germination percentage, light

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[I] INTRODUCTION

Hippophae salicifolia is a deciduous tree restricted to the Himalayan region, between 1500-3500 m a.m.s.l. found in dry temperate forests of western Himalayas in north east aspects, sloppy areas near river banks, sandy soil, and towards sun facing directions.

The seed germination is the prominent reason for regeneration of species in natural habitats. Ecologically, the best germination and growth of species are achieved, where environmental factors are balanced. Light is one of the environmental factors that affect the germination and growth of the plants. Light is important for seed germination and growth. Many species respond to the environment with optimal growth and development according to the light they receive [1]. Some seeds germinate similarly in light and darkness [2], while others do it more readily either under light [3] or dark conditions [4]. Also, light requirements for germination can vary with temperature. It has been demonstrated that some species need a constant temperature and light to germinate and others can germinate either under light or dark conditions but need temperature fluctuations [5]. In other species, stratification [6] or high temperatures [7] replace light requirements for germination

[II] MATERIALS AND METHODS

In the present study, an experiment was designed to assess the effect of different types of light: white (fluorescent) as control, red light with wavelength 630 - 740 nm, green light with wavelength 520 - 570 nm, blue light with wavelength of 450 - 495 nm and yellow light with wavelength of 570 - 580 nm (all electric lights from Philips, India) on seed germination of *H. salicifolia* and also on its radicle and plumule growth.

The investigation was carried out in three Provenances of Uttarakhand State in India viz. Uttarkashi (P1), Chamoli (P2) and Pithoragarh (P3). The geographic range of the provenances selected varied from 30° 03´ to 31° 34´ N latitude, 74° 30´ to 80° 13´ E longitude and 1949 to 3212 m altitude. A minimum of ten trees were randomly selected for collection of seeds from each provenance.

Seed for each replication were placed on top of Whatman no.1 paper in petri plates in the seed germinator at 25 ± 1° C. Each Petri plate was marked with date of experiment and replication number. There were 5 treatments in this experiment including the control (white light). The experiment was undertaken in completely randomized design (CRD) with four replication in each treatment and twenty five seeds per replication.

Data on different germination parameters were recorded after germination at 2-day intervals until no further germination occurred. The seeds were inspected every day and were considered to be germinated when the radicle penetrated the seed coat and attained about 1mm in length [8]. Radicle and plumule length (cm), were measured on the 25th day.

Response Index (RI) was calculated as per the formula given by Richardson and Williamson [9] for the magnitude of inhibition versus stimulation by different lights on seed germination and radicle / plumule

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growth of *H. salicifolia*.

Response Index is calculated as

RI = (T/C -1) × 100
Where,

T = Parameter under Treatment
C = Parameter under Control

[III] RESULTS AND DISCUSSION

The present study revealed that the maximum germination percentage was observed in P2 under red light (89%) followed by yellow light (85%) in P1 and minimum germination observed in green light (50%) in P2. The maximum negative influence was observed in green light (-37.5) followed by blue light (-31.25) in P2. However minimum negative influence was observed in red light (-1.19) followed by yellow light (-4.76) in P3. P3 showed negative influence on seed germination in all the lights under study **[Table -1]**

Table: 1. Effect of different lights on seed germination of *Hippophae salicifolia*

Treatments	Seed germination (%)					
	P_1 (%)	RI	P_2 (%)	RI	P_3 (%)	RI
White	73	-	80	-	84	-
Red	85	16.44	89	11.25	83	-1.19
Blue	64	-12.33	55	-31.25	65	-22.62
Yellow	76	4.11	85	6.25	80	-4.76
Green	58	-20.55	50	-37.5	54	-35.71

Comparing RI = Response Index

The effects of lights on radicle growth showed that maximum radicle length was observed under white (2.08 cm) in P1 and minimum under green light (1.10 cm) in P3. Red ,blue, yellow and green light have negative effect on radicle growth in P1, while in P2 red and green light have negative influence but blue and yellow light have positive influence. In P3 blue and green light have negative influence but red and yellow light have positive influence on radicle growth of *H. salicifolia* **[Table -2]**.

Table: 2. Radicle growth (cm) and Response index (%) of *Hippophae salicifolia* under different lights

Treatment	Radicle growth (cm)					
	P_1	RI	P_2	RI	P_3	RI
White	2.08	-	1.53	-	1.58	-
Red	1.90	-8.65	1.48	-3.27	1.63	3.16
Blue	1.64	-21.15	1.62	5.88	1.28	-18.99
Yellow	2.02	-2.88	1.60	4.57	1.82	15.19
Green	1.15	-44.71	1.20	-2.17	1.10	-30.32

RI = Response Index

The effects of lights on Plumule growth showed that maximum plumule length was observed in yellow (3.34 cm) and minimum under green light (1.13 cm) in P1. Red, blue and green light have negative effect on plumule growth in P1, while yellow light has positive influence. The results of P2 reveal that red, blue, yellow and green light have positive influence on the growth of plumule. P3 revealed positive influence of red, blue and yellow light on Plumule growth of *H. salicifolia* **[Table -3]**.

The results of the study reveal that except in P3, red and yellow light increase the germination percentage **[Table-1]**. However, green light decreases the germination as well as radicle and plumule growth during the study period **[Tables- 2 and -3]**. It is known from earlier works that light is an important factor affecting germination and seedling growth. Research works from Ellis and Robert [10], Hangarter [11], Wapeha and Kaufman [12] and Winslow [13] showed that many plant species responded to the environment with optimal growth and development according to the light they received and Colbach *et al*., [3] reported that some seeds germinated under different lights. In this experiment, the maximum germinations are observed under red light and yellow light irrespective of the provenance. Germinations of seeds of *Ruellia tuberose* [14], *Asteracantha longifolia* [15] and *Cucumis callosus* [16] are also reported to be promoted when

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irradiated with red light. These reports somewhat supported the findings of David and Chawan [15] and Shyam and David [17] that the red region of spectrum (590 and 680µm) was most effective for the germination of light requiring seeds.

Table: 3. Plumule growth (cm) and Response index (%) of Hippophae salicifolia under different lights

Treatment	Plumule growth (cm)					
	P_1	RI	P_2	RI	P_3	RI
White	3.08	-	1.93	-	1.90	-
Red	2.60	-15.58	2.04	5.69	2.00	5.26
Blue	2.46	-20.13	2.16	11.91	2.06	8.42
Yellow	3.34	8.44	2.74	41.96	2.50	31.57
Green	1.13	-63.31	1.10	-43.02	1.22	-35.72

RI = Response Index

H. salicifolia seeds germination started 5-8 days after sowing in red and yellow light and seeds under blue light started to germinate after 12 -15 days. David and Chawan [15] and Shyam and David [17] also reported that the seedling growth of some *Merremia* species was the least in blue light. This was similar to the findings of Wareing and Black [18] and Gwynn and Scheibe [19] with regard to lettuce seeds. *H. salicifolia* seeds under red and yellow light showed the fastest germination. Shyam and David [17] reported that the highest percentage of some *Merremia* sp. was found in red light.

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