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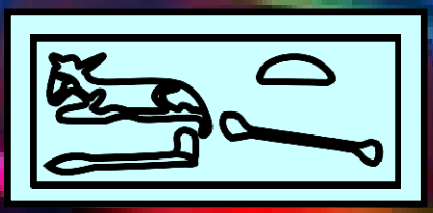

الجمعية المصرية للعلوم الوراثية

PROCEEDINGS OF THE FOURTH INTERNATIONAL CONFERENCE OF GENETIC ENGINEERING AND ITS APPLICATIONS

SHARM EL-SHEIHK SOUTH
SINAI GOVERNORATE
(HILTON - SHARK BAY)

FINAL PROGRAM AND ABSTRACTS

ORGANIZED BY
THE EGYPTIAN SOCIETY OF GENETICS

OCTOBER 5 - 8
2016

الجمعية المصرية للعلوم الوراثية

THE THIRD INTERNATIONAL CONFERENCE OF GENETIC ENGINEERING AND ITS APPLICATIONS

AT SHARM EL-SHEIKH CITY,
SOUTH SINAI, EGYPT
(OCTOBER, 5-8, 2016)

ORGANIZED BY
THE EGYPTIAN SOCIETY OF GENETICS

UNDER THE AUSPICES OF THEIR EXCELLENCIES:

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& LAND RECLAMATION

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The Fourth International Conference of Genetic Engineering & Its Applications

"New Trends in Genetic Engineering & Biotechnology"

Conference Main Topics

Application of Genetic Engineering in:

- ***Plant Production***
- ***Animal, Poultry and Fish Production***
- ***Food and Dairy Industrial Field***
- ***Medical and Pharmaceutical Field***
- ***Genomics, Proteomics and Bioinformatics***
- ***Environmental Biotechnology***
- ***Biosafety and Bioethics of Genetic Engineering***

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CONFERENCE PROGRAM

Wednesday: October 5		
1:00	3:00 pm	Arrive & Lunch
5:00	5:30 pm	Registration
5:30	6:00 pm	Opening session
		Prof. Dr. EL-SAYED H. HASSANIEN **Prof. Dr. MAHMOUD I. NASR** **Prof. Dr. FATTHY ABDEL-TAWAB** **Prof. Dr. EFAT BADR** **Prof. Dr. GHARIB A. GAD EL-KARIM**
6:00	8:00 pm **Chairpersons**	**Plenary Session (Main Hall)** **Prof. Dr. EFFAT BADR** **Prof. Dr. MAHMOUD I. NASR** **Prof. Dr. ABDEL-WAHAB M. HASSAN** 1. From *In Vitro* To The Market. (Taymour Nasr El-Din) 2. Systems Biology Approaches In Infectious Diseases. (Nourtan F. Abdeltawab)
8:00	10:00 pm	**Dinner**

Thursday: October 6		
7:00 am	9:00 am	**Breakfast**
9:30 am	12:30 pm **Chairpersons**	**Session I (Main Hall)** **Prof. Dr. ABDEL-WAHAB I. ALLAM** **Prof. Dr. IBRAHIM EL-SHAWAF** **Prof. Dr. EMAN M. FAHMY** 1. Somaclonal Variation In Bread Wheat (*Triticum Aestivum* L). Vi- Assessment Of Genetic Diversity Among Advanced Somaclones Using Agronomical, Cytological And Molecular Markers. K. Z. Ahmed, S. A.-M. Osman, R. A. Ragab And K. S. Mortada. 2. Bisecting N-Glycan Structure Promotes Ovarian Cancer Stem Cells Malignant Behavior. Heba Allam And Karen Abbott. 3. Estimation Of Genetic Variability, Heritability, Genetic Advance And Rapd Analysis Of Sesame Populations Under Water Deficit Environment. Tarek Youssef Bayoumi, Eman T. Abdou, M. Sabry H. Yousef And M. A Emam. 4. A First Genetic Analysis Of A Lessepsian Species As A Tool For Predicting The Invasion Force Of A Potential Economic Resource. T. A. Temraz, F. Denis, W. K. B. Khalil, J. Ben Souissi And F. Kh. Abdel-Gawad. 5. Molecular Ecology Unfold The Effect Of Climate Changes On Plants Phenotypic And Genotypic Plasticity. Mahmoud Magdy And Mohamed Farag.
12:30	1:30 pm	**Poster Session**
1:30	3:00 pm	**Lunch**
5:00 am	7:00 pm **Chairpersons**	**Session II (Main Hall)** **Prof. Dr. ABDEL-WAHAB M. HASSAN** **Prof. Dr. EKRAM SALAH EL-DIN** **Prof. Dr. AHMED ABO-DOMA** 1. Genetic Transformation Of Egyptian Commercial Barley (*Hordeum Vulgare* L.) Cultivars Giza 123 And Giza 125 Using Particle Bombardment. Ebtissam Hussein, M. Sakran, A. Haider, A. El-Wassief And M. Hazman. 2. Detection Of Some Environmental Pollutants By Functional Toxicogenomic Analysis. Heba H. Hassan, F. M. Abdel-Tawab, Alia El Seoudy And K. F. Ebn El-Walid 3. Genetic Relationships Of Some Egyptian Wheat Cultivars (*Triticum aestivum* L.) Through Internal

		Transcribed Spacers And 5.8 S rDNA Sequences. Lamyaa M. Sayed. 4. Response Of Heat Shock Gene(S) Expression In *D. melanogaster* To Silver Nanoparticles Treatment. Marwa R.Mahmoud, Naglaa M. Ebeed, F. M. Abedl-Tawab And Nermeen M.Abedl-Gawad. 5. Genotoxicity Of Ethidium Bromide In Albino Mice Treated With Bacterial Probiotic. Hala M. Zoghly, M. A. Rashed, R. A. Ali, Nermin M. Abd El-Gawaad, And M. Magdy..
7:00	8:00 pm	**Poster Session**
8:00	10:00 pm	**Dinner**

Friday: October 7		
7:00	9:00 am	**Breakfast**
10:00	12:00 am Chairpersons	**Closing Session (Main Hall)** **Organizing Committee** Recommendations & Farewell Address

ABSTRACTS

FROM *IN VITRO* TO THE MARKET

TAYMOUR NASR EL-DIN

Micropropagation is true – to – type propagation of selected or elite genotype using *in vitro* tissue culture techniques. Most often micropropagation methods are also associated with mass production at competitive price in the market place. The ultimate goal of the commercial tissue culture operation is to gain profit, it is the case with any economic activity in other fields. From commercial point of view, one has to look carefully to some fundamental aspects to be successful. Financing of the project, knowledge of the market and its access, research development program and production management are essential for commercial tissue culture laboratory.

The majority of micropropagated plants in US (1980) are marketed in ornamental segment of horticulture industry (flowers and foliage plants).In Egypt , major crops commercially produced nowadays by tissue culture technique are banana ,potato, strawberry, ornamental plants and date palm. More than 12=14 million plantlets per year produced throughout few local large companies.

Increased use of *in vitro* plants in future will depend on several factors:

1- The cost should be competitive with conventional methods of propagation
2- The micro propagated plant must be adaptable with cultural practices employed by consumer or farmer
3- The product must be genetically stable and phenotypically identical to the original stock plant
4- The micro propagated plant must be produced at the proper time with correct quantities to meet consumer orders and needs

Biotechnology is only part of modern process for developing agriculture commodities particularly in developing countries besides the conventional plant breeding methods .The commercialization of tissue culture is one of promising opportunists in the field of agriculture.

SYSTEMS BIOLOGY APPROACHES IN INFECTIOUS DISEASES

NOURTAN F. ABDELTAWAB'S TALK TITLE

Clinical outcomes of infectious diseases are controlled by complex interactions between the host and the pathogen. Systems biology approaches offer an unbiased holistic approach to understanding the basis of differential responses to diseases. We applied systems biology approach to studying invasive Group A streptococcal (GAS) infections and sepsis using advanced recombinant inbred (ARI) panel of mice. We assessed several traits associated with differential host responses to GAS sepsis, and analyzed variations in these traits in the context of mice genotypic variability. This allowed us to map quantitative trait loci (QTL) associated with modulating susceptibility to severe GAS sepsis on chromosome (Chr) 2 and Chr X. Based on linkage analyses, gene ontology, co-citation networks, and variations in gene expression, we identified interleukin 1 (IL1) and prostaglandin E (PGE) pathways as prime candidates associated with modulating the severity of GAS sepsis. In conclusion, we found that variations in the severity of GAS sepsis have a strong biology component that is complex and multigenic. Our overall approach of systems genetics, where we systematically dissected genetic, molecular, cellular and functional differences that may be associated with differential host susceptibility to GAS provided us with tremendous insight into disease mechanism. The knowledge gained can help in the development of better diagnostics and means to predict disease severity. Prediction of response based on a set of genetic and prognostic biomarkers will help customize patient care providing a basis for personalized interventions.

ESTIMATION OF GENETIC VARIABILITY, HERITABILITY, GENETIC ADVANCE AND RAPD ANALYSIS OF SESAME POPULATIONS UNDER WATER DEFICIT ENVIRONMENT

T. Y. BAYOUMI, EMAN T. ABDOU, M. S. H. YOUSEF AND M. A EMAM

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ABSTRACT

Water deficit is a common adverse environmental condition that limits plant growth of Sesame. Information on the genetic diversity in sesame is limited, in addition to, genotypes that may be considered drought tolerant. Therefore, a field trial was conducted for two successive seasons to evaluate four populations of sesame for drought tolerance. The principle of this research is to analyze the genetic structure of sesame to revise of genetic variability, heritability, genetic advance and RAPD molecular marker were undertaken at experimental farm; faculty of agriculture; Suez Canal University: Ismailia, Egypt under three water treatments. The magnitudes of phenotypic coefficient of variation values for all traits were higher than the corresponding values Phenotypic coefficients of variability and heritability Estimates ranged from …
Phenotypic coefficients of variability (PCV) ranged from 7.60 to 34.34% and

(GCV) ranged between 5.58% for all the traits were higher than the corresponding values and broad sense. The development of molecular markers for physiological traits has made significant headway in recent years with the advancement of new technologies. Consequently, in our study the use of molecular markers; RAPD technique with 9 primers was

detected 91 polymorphism alleles for the genotypes with 79.12% polymorphism. The most Polymorphic Information Content (PIC) value and polymorphism percentage was detected by OPA-07primer that showed the high score from bands 13 with polymorphism 69.23%. While, OPO-19 revealed low level from bands was 6 with percentage 83.33%. Also, OPA-02, OPA-04 and OPO-13 revealed 9 fragments with 77.78% polymorphism. While, primers OPB-07, OPB-10 and OPO-14 showed 11 bands with 81.82% polymorphism. The last primer revealed 12 bands with 75% polymorphism. Therefore, these recently developed techniques could be enable faster identification and characterization of drought-related gene(s).

SOMACLONAL VARIATION IN BREAD WHEAT (*Triticum aestivum* L). VI- ASSESSMENT OF GENETIC DIVERSITY AMONG ADVANCED SOMACLONES USING AGRONOMICAL, CYTOLOGICAL AND MOLECULAR MARKERS

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ABSTRACT

Wheat (*Triticum aestivum* and *T. durum*), is the most important cereal crops in Egypt which cover more than 1.42 million Ha with an annual production of 9.46 million tons (FAO 2013), that supply only 55 % of the fast growing population demand. Therefore, Egypt is the biggest producer for wheat in Africa, but also, it is the biggest importer worldwide. Thus wheat occupies a unique position in the Egyptian agricultural economy. There is essential needed to improve wheat productivity to meet our food requirements. Using classical breeding methods hampered by several limitations, therefore, new biotechnology ways open new facilities to overcome these limitations.

Somaclonal variations are the genetic variability present among cultures somatic cells and their derived plants. Our 31 somaclones (at their R8 and R9 generations) were evaluated at agronomical, cytological and molecular levels; these somaclones are derived from 4 Egyptian and 2 foreigner bread wheat cultivars (*Triticum aestivum* L.). Significant differences between parental cultivars and its somaclones for yield and yield components characters were observed. Some somaclones were superior genotypes comparing with their parents and/or other tested

genotypes. S8-17 somaclone had the highest value for most tested agronomic traits comparing with other 18 S8-derived somaclones and parental Sakha 8 cultivar.

The meiotic behaviour of PMCs of somaclones was shown many types of chromosomal abnormalities i.e. chromosome laggard, chromosomal bridges, stickiness, micronuclei, uni- & multi-valents and outside chromosome. Significant differences in abnormal PMCs between the parental cultivars and its somaclones in both 8^{th} and 9^{th} generations were detected in some cases. Almost all examined PMCs have regular 21 bivalents and most of them were predominantly of ring shape. Also, S8-17 somaclone showed cytological stable as compared with the parental S8 cultivar.

RAPD and ISSR molecular markers were used to check the genetic diversity of 6 parental cultivars and their 12 somaclones. The tested 5 RAPD primers generated 38 amplified DNA fragments (141 to 920 bp), while, the 5 ISSR primers generated 43 DNA fragments (134 to 718 bp). Wide range of monomorphic and polymorphic DNA fragments were generated with different primers and wheat genotypes. The combine (RAPD + ISSR) dendrogram showed closely matching results in distinguishing the wheat cultivars and their somaclones according to their genetic background and geographic origin.

GENETIC TRANSFORMATION OF EGYPTIAN COMMERCIAL BARLEY (*Hordeum vulgare* L.) CULTIVARS GIZA 123 AND GIZA 125 USING PARTICLE BOMBARDMENT

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1. Genetics Dept., Faculty of Agriculture, Cairo University, Egypt
2. Organic Chemistry Dept., Faculty of Science, Tanta University, Egypt
3. Botany Dept., Faculty of Science, Tanta University, Egypt
4. Biochemistry Dept., Faculty of Science, El-Mansora University, Egypt
5. Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt

ABSTRACT

Transformation of immature embryo derived calli of the two barley cultivars Giza 123 and Giza 125 was performed with the biolistic particle delivery system using the plasmid pAHKD10 (harboring the methionine rich 10kDaY DNA fragment) and the plasmid pAB8 (harboring the *bar* gene). Different transformation parameters were assayed, i.e. the age of calli (six and ten days-old), the acceleration pressure (900, 1100 and 1350 psi) and distance between the rupture disk and target tissue (5, 8 and 11 cm). The highest average number of bialaphos resistant calli and putatively transgenic plantlets were obtained from the two barley genotypes when bombarding ten-days-old calli at an acceleration pressure of 1100 psi. Stable transformation was confirmed in T_0 transformed plants by means of leaf painting with the herbicide Basta, PCR analysis using primers specific for the *bar* and 10KDaY genes and Southern blot hybridization using specific probe.

A FIRST GENETIC ANALYSIS OF A LESSEPSIAN SPECIES AS A TOOL FOR PREDICTING THE INVASION FORCE OF A POTENTIAL ECONOMIC RESOURCE

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[6] *Department of Water Pollution Research, Center of Excellence for Advanced Science, National Research Center, 33 Buhouth St, 12622 Dokki, Giza, Egypt*

ABSTRACT

Populations of *Fulvia fragilis* collected from native area (Red Sea, Egypt) and from colonized area (Gulf of Tunis, Tunisia) were studied using molecular genetic tools. The molecular approach allowed us to characterize a genetic signature of this non-native species. These data are indispensable tools to clarify the taxonomic position of samples taken from both sides of the Suez Canal (Mediteranean and Red Seas). The analysis of nuclear and mitochondrial genetic markers provided a first assessment of the genetic diversity of this bivalve in its area of origin and settlement area, as well as led to estimate the impact of colonization of the Mediterranean on the genetic diversity intrapopulation of a lessepsian species.

MOLECULAR ECOLOGY UNFOLD THE EFFECT OF CLIMATE CHANGES ON PLANTS PHENOTYPIC AND GENOTYPIC PLASTICITY

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2. *Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt.*

ABSTRACT

Climate changes form part of the natural environmental changes of the planet earth. Through millions of years, climate changes contributed significantly in the biodiversity, creating combined bioclimatic conditions affecting both biotic and abiotic factors that contributed to the population dynamics (e.g. selection, mutations and gene flow), resulting in adaptive genetic variation that can reach to adaptive speciation. Since the industrial revolution and the consumption of fossil fuels as a source of energy, CO2 emissions had increased dramatically causing the acceleration of the natural climate change rapidly to sever limits that disturbs the adaptive variation progress and eventually lead to the extinction of a species. Under such circumstances, a plant species may be vulnerable with high risk of extinct or may undergo genotypic and phenotypic adaptive variation to increase its probability to survive the changes.

Molecular biologists, specialized in molecular genetic techniques were those with the potentials to explore the genetic variation on molecular levels, combining their knowledge with ecologist and evolutionary biologists were able to correlate and understand the selection and migration signatures in a genetic pool of a threaten or endangered species due to environmental and climate changes which formed what is currently known as “Molecular Ecology”. Many important cases of study proven the efficiency of such new field, and contributed greatly to conserve and overcome the vulnerable state of several plant species.

As a prospective application, molecular ecology is useful for crop conservation and breeding, using climate prediction modelling, breeding program can be directed toward climate-related traits (e.g. higher temperature tolerance, changes in soil salinity, and changes in humidity levels). Tissue culture integration as a tool to stimulate predicted scenarios of climate changes for early selection of important crops propagated in-vitro.

The expanding and ever upgrading toolbox available to molecular ecologists holds a promising tool for identifying many traits relevant to species fitness under global change.

BISECTING N-GLYCAN STRUCTURE PROMOTES OVARIAN CANCER STEM CELLS MALIGNANT BEHAVIOR.

HEBA ALLAM[1,2] AND KAREN ABBOTT[1]

1. *University of Arkansas for Medical Sciences, Department of Biochemistry and Molecular Biology, AR, USA*
2. *National Liver Institute, Menoufiya University, Department of Microbiology and Immunology*

ABSTRACT

Understanding the biology of sphere forming cells may contribute to the identification of novel therapeutic targets for metastatic Epithelial Ovarian Cancer (EOC). Emerging data indicate that cancer stem–like cells contribute to chemoresistance and poor clinical outcomes in many cancers, including ovarian cancer. N-Acetylglucosaminyltransferase III (GnT-III) is generally regarded as a key glycosyltransferase in N-glycan biosynthetic pathways. In this study, we tested the hypothesis that the bisected N-glycan structure catalyzed by GnT-III is crucial for maintenance of Ovarian cancer stem cell properties by evaluating the effects of knocking down GnT-III expression, as defined by expression of the cancer stem–like markers, stem cell-related genes like CD133, CD44 and NOTCH. The OVCAR-3 cell line showed a significant decrease in cell proliferation following knockdown of GnT-III. OVCAR-3 parent cells formed a greater number of spheroids compared to SiGnT-III cells, which demonstrated limited survival in anchorage-independent conditions by grouping together to formed loosely adhesive cell clusters. Moreover, serial passaging indicated that OVCAR-3 parent cells maintained their sphere- forming ability over multiple passages, whereas the number of cell aggregates formed by SiGnT- III cells was lost after passage 3. Furthermore, we found that the expression of GnT-III and glycosylation of periostin (POSTN), a known acceptor for GnT-III glycosylation, increases Notch receptor protein levels and Notch activity. Suppression of

GnT- III reduced activated Notch receptor levels (NCID). These findings provide important insights into the functional role of bisecting glycans in ovarian cancer and suggest that GnT-III may therefore represent a potential new target for preventing tumor invasion and metastasis.

STUDY OF MULTI-DRUG RESISTANCE IN *Serratia marcescens* CLINICAL AND ENVIRONMENTAL ISOLATES IN EGYPT

ASSEM M. ABD EL-NABY[1], NOURTAN F. ABDEL TAWAB[2], MAHMOUD F. DARWISH[1], MAGDY A. AMIN[2]

(1) Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University-Assuit branch, Assuit, Egypt
(2) Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Kasr El-Aini, Cairo, Egypt

ABSTRACT

Serratia marcescens, a Gram-negative bacillus, was previously known as an innocuous, non-pathogenic microorganism. Recently, *S. marcescens* has been isolated from hospitals and found to be a causative agent of bacterial endocarditis. *S. marcescens's* ability to cause infection was once thought to be limited to patients with chronic debilitating disorders. Nosocomial infections caused by this organism are often hard to treat because of both the intrinsic resistance of this species and its abilities to acquire further resistance to multiple groups of antimicrobial agents. *S. marcescens* usually produces prodigiosin, an alkaloid responsible for the red pigmentation. Prodigiosin has been found to have antibacterial, antifungal, antiprotozoal, antimalarial and even anticancer activities. Although many activities have been associated with prodigiosin, the physiological role of this pigment in the producing bacteria is not fully understood. Studies associating expression of virulence factors with pigment production suggested that pigment is repressed by some virulence factors. However, the exact association of pigment with multi-drug resistance in clinical isolates is still unclear. The overall aim of our study is to understand the association between pigment

production and virulence in environmental and clinical isolates. We collected 22 isolates of *S. marcescens*, four environmental and 18 clinical isolates from cancer institute and ICU in Assuit university hospital. From these, a total of eight isolates were pigmented, four from environmental isolates and four from clinical isolates. The isolates were identified by standard conventional biochemical tests and their antimicrobial susceptibilities were determined by disk diffusion method and the results were interpreted according to the Clinical and Laboratory Standards Institute. The antibiotics used were β-lactams, aminoglycosides and fluoroquinolones. Most of isolates showed marked resistance toward β-lactams antibiotics but they were very sensitive for fluoroquinolones. The resistances toward β-lactams antibiotics suggest that the isolates might posses β-lactamases genes e.g. (blaSHV –blaTEM –blaCTX-M) and/or AmpC genes. Our future studies include genetic basis of multi-drug resistance and it's relation to pigment and virulence factors. In addition, we will perform phylogenetic analysis of selected clinical and environmental isolates and identification of chromosomal and plasmid-encoded genetic determinant of multi-drug resistance.

DEVELOPMENT OF AFLP, ISSR AND RAPD MARKERS RELATED TO HIGH-YIELD COMPONENT TRAITS IN JOJOBA

MOHAMED A.M. ATIA, SHAFIK D. IBRAHIM, NAHLA A. AWAD AND SAMI S. ADAWY

ABSTRACT

Simmondsia chinensis popularly known as jojoba, a monogeneric dioecious shrub of the family *Simmondsiaceae* from arid zones, has emerged as a cash crop in India and abroad. The plant is important to commerce as its seeds store liquid wax (40–60% by dry weight). Due to wide variations in yield, selection of female superior plants is the most important parameter for increasing yields in future populations. In this study, based on the evaluation of fifty female strains for 13 traits representing morphological, seeds characteristics and yield traits, six jojoba strains represent the extremes strains for yield and seed weight traits were selected for molecular analysis. The correlation coefficient results revealed that highest positive correlation was observed between average leaf area and yield (0.65) and the lowest positive correlation was observed between average seed length and yield (0.14). While, highest negative correlation was found between average seed diameter and yield (-0.38), and the lowest negative correlation was observed between average seed weight and yield (-0.07). For molecular markers analysis, the selected strains were characterized using 8 AFLP, 16 ISSR and 30 RAPD primers/primer combinations. For yield, the AFLP, ISSR and RAPD produced 531, 138 and 325 total scorable bands with percentage of polymorphism 28.0, 35.5 and 35.6%, respectively. While, for seed weight, they generated 524, 135 and 317 total scorable bands with percentage of polymorphism 27.0, 31.1 and 34.0%, respectively. A

dendrograms based on UPGMA analysis of AFLP, ISSR, RAPD and combined data were constructed for both yield and seed weight extremes. For yield, all dendrograms successfully grouped the superior strains in one cluster with high degree of similarities. While, for seed weight all dendrograms successfully grouped the superior strains in one cluster except ISSR dendrograms. For yield, 1 AFLP PC, 3 ISSR primers and 6 RAPD primers successfully identified 1, 4 and 7 unique positive markers, respectively. In addition, 2 AFLP PCs, 4 ISSR primers and 8 RAPD primers identified 4, 4 and 8 unique negative markers, respectively. For seed weight, 4 AFLP PCs, 2 ISSR primers and 5 RAPD primers successfully identified 13, 3 and 5 unique positive markers, respectively. Moreover, 6 AFLP PCs, 2 ISSR primers and 3 RAPD primers identified 14, 2 and 5 unique negative markers, respectively. This results represent the first case study combining different molecular marker techniques, in addition to agronomical and morphological evaluation of 13 traits, in order to development unique positive and negative markers can be used to identify superior jojoba strains in early stages.

DIFFERENTIAL GENE EXPRESSION RELATED TO DROUGHT TOLERANCE IN BARLEY (*Hordeum vulgare* L.)

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2. *Genetics Dept., Fac. Agric., Ain Shams Univ., Shoubra El-Kheima, Cairo, Egypt.*

ABSTRACT

Differential display-polymerase chain reaction (DD-PCR) technique was used to analyze differentially expressed sequence tags (ESTs) in barley (*Hordeum vulgare* L.) cultivar Giza 126 under drought stress. An array of 66 differentially expressed EST and 74 EST fragments were obtained from roots and shoots, respectively. The sequences of these fragments were identified and determined using a bioinformatics approach; BLAST. Results of the database sequence alignment for roots identified 28 (42%) fragments showed homology with some predicted proteins, 12 (18%) fragments showed homology with predicted proteins under abscisic acid (ABA), 7 (11%) fragments showed homology with predicted proteins under low temperature, 10 (15%) fragments showed homology with transcription factors, 5 (8%) fragments showed homology with different enzymes, 3 (5%) fragments showed homology with un-identifying clones and one (1%) fragment showed homology with LEA protein. With regard to shoots, 34 (46%) fragments showed homology with some predicted proteins, 13 (18%) fragments showed homology with predicted proteins under abscisic acid (ABA), 4 (5%) fragments showed homology with predicted proteins under low temperature, 4 (5%) fragments showed homology with transcription factors, 7 (10%) fragments showed homology with different enzymes, 8 (11%) fragments showed homology with un-identified clones, 3 (4%) fragments showed homology to different genes and one (1%) fragment showed homology with 14-3-3 protein. These results could be used to improve abiotic stress tolerance of economic crops.

BIODIVERSITY OF GUT MICROFLORA OF *Oreochromis niloticus* BASED ON CULTURE-INDEPENDENT rRNA GENE ANALYSES AT LAKE NASSER, EGYPT

MAI A. WASSEL[1], HOSAM E. ELSAIED[1*] AND MOHAMED ABDEL-SALAM RASHED[2]

1. *Genetics and Genetic engineering research group, National Institute of Oceanography and Fisheries, NIOF, Cairo, Egypt.*
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ABSTRACT

The diversity of *Oreochromis niloticus* gut microbiome domains, eukaryotes, bacteria and archaea, was studied to understand the contribution of microbiota to the health of the fish. Fishes were collected from four different Khors, Kalabsha, Wadi Abyad, Tushka and Korosko, of Lake Nasser, Egypt. The approach of this study depends on culture-independent PCR/DGGE and sequence of small subunit of rRNA genes, 18S rRNA gene and 16S rRNA gene. The DGGE patterns displayed 5, 12 and 5 band groups, phylotypes, for eukaryotic 18S rRNA gene, bacterial and archaeal 16S rRNA genes, respectively, in gut contents from the studied khors. DGGE showed bands, which were common and specific for each site and could be used as a bar code to certify the origin of the fish. Statistical analyses, using binary matrix, showed numbers of DGGE bands, 1, 2 and 2, for eukaryotes, bacteria and archaea, respectively, were commonly occurred in all studied khors. The DGGE phylotype, 3.Euk.Kr characterized eukaryotes in Khor Korosko. Phylogenetic analyses showed that two of eukaryotic phylotypes, 1.Euk.Kl.Kr and 2.Euk.Common, were belonged to crustacean Ostracoda. Bacterial phylotypes in all studied khors were located in the branch of cyanobacteria, alphaproteobacteria, but most of them constituted unique phylogenetic lineages within the branch of uncultured environmental bacteria. All

archaeal phylotypes were located in the branch of methanogenic uncultured euryarchaeota. Some helminthes, of the genera *Neoechinorhynchus* and *Catenula*, -like rRNA gene phylotypes were recorded in guts from Kalabsha, Tushka and Korosko, suggesting common gut parasitic worms. The DGGE patterns and sequence analyses showed high similarities of eukaryote, bacteria and archaea rRNA gene phylotype compositions in fish guts from distant khors, implicating core gut microbiome. This is the first survey of all microbiome domains in tilapia guts at Lake Nasser based on molecular approaches.

DETECTION OF SOME ENVIRONMENTAL POLLUTANTS BY FUNCTIONAL TOXICOGENOMIC ANALYSIS

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ABSTRACT

Many chemicals are added to foods to storage life or to enhance color, flavor, and texture. Food additives must be cleared by the US Food and Drug Administration (FDA) before being allowed into the food supply, and thorough testing is done in lab animals to determine any effects on cancer as part of this process. Sodium Benzoate and Butylted Hydroxyanisole (BHA) are common additives which are usually present in very small quantities in food, and some are nutrients that may have beneficial effects. *Saccharomyces cerevisiae* is useful model for testing recommended concentrations of food additives because of its similarity with human genome that reachs up to 46% and could therefore, confer the expression of genes related to human cancer cells.A set of yeast knockout strains representing a wide range of deleted genes were subjected to different concentrations of each additive. Comparison between data obtained from *S. Cerevisiae* with those resulting from different human cancer cell lines revealed significant deletarious effects of the tested food additives on human health.

GENETIC RELATIONSHIPS OF SOME EGYPTIAN WHEAT CULTIVARS (*Triticum aestivum* L.) THROUGH INTERNAL TRANSCRIBED SPACERS AND 5.8 S rDNA SEQUENCES

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ABSTRACT

Thirteen cultivars of egyptian wheat (*Triticum aestivum* L.); Giza 168, Seds 1, Seds 12, Seds 13, Gemmeiza 7, Gemmeiza 9, Gemmeiza10, Gemmeiza 11, Sahl 1, Shandaweel 1, Sakha93, Sakha 94 and Sakha 69 were used to study the efficiency of transcribed region, internal transcribed spacers (ITS) which were found on either side of 5.8S rRNA (rDNA) gene and were described as ITS1 and ITS2. The length and sequences of ITS regions of rDNA repeats may vary because they are believed to be fast evolving. This makes the ITS region an interesting subject for evolutionary/phylogenetic investigations as well as biogeographic investigations. Already, the phylogeny tree of the 14 nucleotides sequence was done using the Clustal Omega free program, which divided the cultivars into three main clusters; Cluster I contains two subcluster, the first one included the sequence from NCBI sit (GenBank: FJ609737.1) with Shandaweel 1, Sakha 94, Gemmeiza 9, Gemmeiza 10, Gemmeiza 7 then Gemmeiza 11 then Sids 13, while the second subcluster was Sahel 1 and Sakha 69. Cluster II contain Sakha 93 with Sids 1, whereas cluster III encompass Sids 12 and Giza 168. From the obtained results, internal transcribed spacers (ITS) proved to be an efficient method to measure the relationship between even closed cultivars.

RESPONSE OF HEAT SHOCK GENE(S) EXPRESSION IN *D. melanogaster* TO SILVER NANOPARTICLES TREATMENT

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ABSTRACT

The heat shock proteins (HSPs) that are abundantly expressed in insects are important modulators of insect survival and used as sensitive biomarkers for xenobiotics. Increased nanomaterial production and their wide range of applications imply a higher risk of human and environmental exposure including silver nanoparticles (AgNPs).

The aims of the present study were to investigate the responses of *D. melanogaster* to exposure to AgNPs with regard to changes in the expression of heat shock proteins *hsp* genes (*hsp23*, *hsp26*, *hsp27* and *hsp60*) and to assess the correlations between *hsps* genes, antioxidant systems and oxidative stress. Also to find one or more genetic markers that can predict biological stress in fruit flies. Characterization of AgNPs by using transmission electron microscopy (TEM) and dynamic light scattering (DLS) analysis revealed agglomeration of silver particles in water, spherical shape, and uniform size with an average diameter of between 15-70 nm in diameter and the hydrodynamic diameters were 74.35 nm.

Upon larvae exposure to different concentrations of AgNPs, significant changes were determined in body color and toxic effects such as melanization, necrosis and malformations and the larvae failed to pupate at high concentrations. The acute toxic effect of AgNPs on *D.*

melanogaster was observed for the AgNPs concentration equal to 1600 µg/ml. At this silver concentration, 50% of the tested flies were unable to leave the pupae, and they did not finish their developmental cycle.

Antioxidant activity by DPPH scavenging assay of drosophila larvae consumption of AgNPs resulted in significantly decreased activity of antioxidant. Meanwhile, AgNPs promoted reactive oxygen species (ROS) generation.

Quantitative real-time PCR (qRT-PCR) analysis of HSP genes expression revealed that the analyzed markers responded significantly to 1600 µg/ml of AgNPs and induced oxidative stress. AgNPs up-regulated the expression of heat shock *hsp26* and *hsp27* genes whereas the relative expression of *hsp60* showed a slight increase in its expression. In contrast, HSP23 expression decreased. Finally, *Drosophila*, was considered an established genetic model system, can be well utilized for further understanding of the biological effects of nanoparticles.

CLONING AND FUNCTIONAL ANALYSIS OF AN OsCIPK15 UPSTREAM REGULATORY REGION IN WHEAT, AND ITS POSSIBLE MANIPULATION FOR USE AS CONSTITUTIVE AS WELL AS SALT-INDUCIBLE PROMOTER

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ABSTRACT

A major mechanism of stress tolerance in plants is via increasing the cytosolic Ca^{2+} ion concentration, which acts as a secondary messenger to transduce the cellular responses to the extracellular environment. The involvement of different types of Ca^{2+}-sensing protein kinases, such as CIPK [Calcineurin B-like (CBL) protein Interaction Protein Kinase], in stress responses, has been investigated in different plants In this study, we isolated and characterized an upstream regulatory promoter from an Egyptian rice cultivar, (*Oryza sativa* cv Giza182, and tested its ability to drive the expression of β- glucuronidase (*gus*) reporter gene in wheat. A 1275-bp DNA fragment upstream of the ATG translation initiation codon of the OsCIPK15 gene, referred in this study as UP-CIPK15, was cloned, sequenced and analyzed for stress-related cis acting elements. This DNA fragment was fused to the GUS reporter gene, with and without the Act-1 intron (the first intron of rice actin 1 promoter). The fusion cassettes were subcloned in a promoterless pAB6 plant expression vector and the recombinant plasmids were transformed into wheat mature embryo for transient expression. Our results revealed that, UP-CIPK15 with the Act-

1 intron (UP-CIPK15/Act-1) was induced by salt, whereas, UP-CIPK15 without the Act-1 was constitutive. Furthermore, the expression level of GUS using UP-CIPK15 was comparable, if not more, than CaMV35S promoter.

Data from this work suggests that UP-CIPK15 can be used as an alternative promoter candidate for high levels of constitutive and/or inducible expression.

GENOTOXICITY OF ETHIDIUM BROMIDE IN ALBINO MICE TREATED WITH BACTERIAL PROBIOTIC

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ABSTRACT

Ethidium bromide (EtBr), is a nucleic acids intercalating agent used extensively as a fluorescent dye in molecular genetics laboratories. The current study aimed to determine the potential genotoxicity of EtBr on mammalian tissue through phago and/or pinocytosis, and to investigate the antigenotoxic effect of probiotic bacteria (Lactic Acid bacteria), using albino mice as a model of study.

Mice were randomly divided into 7 groups with 7 different transactions, in which different doses of EtBr were used individually as drinking solutions with and without probiotic bacteria, which was introduced as a single dose in form of food additive. After one month of the treatments, liver was tested using histological assay, DNA fragmentation analysis and semi-quantitative RT-PCR technique.

No significant genotoxic effect for EtBr on liver was recorded on both histological examination and DNA fragmentation analysis, despite the fact of significant increase in the expression of TP53 gene over the negative control group correlated with the dosages increase. However, the TP53 expression was altered upon applying the probiotics, while some histological changes were detected, but no DNA fragmentation was detected.

The seriousness of EtBr on the organism health is conditional with the applied doses. Probiotics was not luckier than ethidium bromide, it did not provide expected health benefits. This unexpected action of probiotic may be due to the used dose, and how to use it. Reasons to explain such paradox is to be tested further on both histological and molecular level using multiple controls and integrative experiments.

POPULATION STRUCTURE OF THE REDBELLY TILAPIA AMONG CLOSED AND OPEN FRESH WATER SYSTEMS OF EGYPT

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ABSTRACT

The redbelly (*Tilapia zillii* L.) is one of the native tilapiine of the fresh water systems of Egypt. Known to be uneconomically important due to its low growth rate but environmentally tolerant to several environmental conditions. Thus, is more adaptable to mostly every water source in Egypt including sources with high salinity conditions, which some current investigators are targeting the fish to improve its growth rate up to economical levels. The current study aimed to genotype random samples from different open and close fresh water systems in Egypt, to define its population structure and possible gene flow among sampled locations. Small subunit rDNA (18S), Large subunit rDNA (28S) and cytochrome oxidase subunit I (COI) were sequenced for 4 samples per each location (Nasser Lake, Qaroun Lake, Borolous Lake, Serw canal, Qanater reservoir, Suez Canal, Ismailia's aquafarms, Ismailia canal) using universal primers.

According to the 18S and 28S, sampled species was confirmed to be all redbelly *T. zillii*, were no cross hybridization was found with another relative species (e.g. *Oreochromis niloticus*and/or *Sarotherodon galilaeus*), such genetic regions are highly conserved among species, in which speciation due to location differences in nature and environmental condition is not occurring. In the case of COI, high genetic polymorphism

was detected, 7 haplotypes were found among 32 samples from 8 locations. Phylogenetic signal showed three haplotypes groups X (4 haplotypes), Y (unique haplotype) and Z (2 haplotypes). The most abundant haplotype group (X) appeared in all 8 locations, while haplotype group Y appeared only within the Nile stream (Nasser lake at south, Qanater reservoir in the middle and Borolous lake at north). Haplotype group Z appeared in Ismailia's aquafarms and Borolous lake only.

Following the natural connection among samples locations, haplotype Y showed a limited distribution outside the Nile stream, in contrast haplotype X appeared in all locations with and without a direct connection to the Nile stream. Our results suggest that X group possess an adaptive variation to be distributed among all locations successfully even those with high salinity levels (Qaroun Lake, Serw canal, Suez Canal, Ismailia's aquafarms, Ismailia canal) unlike haplotype group Y. Using Nile tilapia juvenile from Borolous lake to Ismailia's aquafarm would be a suitable explanation for the presence of haplotype group Z that hitchhiked in the process. Interestingly haplotype Z was reported previously from water system in Israel as a dominant haplotype, in contrast to X haplotypes, while Y was never reported.

The current study gave a scope on the population structure of redbelly tilapia and its genotypes in the Egyptian environment. The current study recommends considering the variation among sampling candidates for any salinity-related breeding program.

MICROBIAL SURVEY AND MOLECULAR IDENTIFICATION OF BACTERIA AND FUNGI ISOLATED FROM OF DJOSAR PYRAMID THE MOST ANCIENT HERITAGE PYRAMID IN EGYPT

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ABSTRACT

Stone surface are complex environments consisting of several microhabitats, which enable a diverse range of microorganisms to proliferate in spite of the harsh conditions resulting from low water availability and nutrient concentration. some use an enzymatic activity to inhibit and decay stones and rocks for nutrients. As well, stone structures and monuments are vulnerable to such organisms causing what is known as bio-deterioration. In Egypt, archeological sites and prehistoric stone monuments represents an important part of the cultural heritage and national income, especially pyramids in which that had never been reported for a microorganism survey, however a primary study on oldest stone monument in the world "Djosar pyramid". It is considered as the first Egyptian pyramid and known as the oldest building in the world built entirely of stone.

This study was to detect and isolate the existent microbial communities within Djosar Pyramid area, that is considered to be a new examining site that never been reported.13 rocks and sand Samples were collected from the entrance of Djosar pyramid and surrounded area using sterile material then was powdered, mortar and suspended (1:10) in saline

0.001% tween 80, for the isolation and the enumeration of cultivable chemoorganotrophic microorganisms, one ml- volumes of suspension were inoculated in duplicate to specific media for Bacteria. BRII and GEO media and DRBC Dichloran Rose Bengal Chloramphenicol agar (DRBC; King *et al*. 1979; Urzi *et al*. 1992) and Potato Dextrose agar PDA for fungi to obtain pure isolates. DNA was extracted from both bacteria and fungi isolates, PCR was performed using universal 16S and ITS primers for bacteria and fungi respectively. Successful amplicons were sequenced and analyzed using bioinformatics software and tools.

The micro flora that found on the stone surfaces is variable and includes Black fungi, Actinomycetes and other bacteria of various phylogenetic affiliations. 17 isolates of Bacteria were identified using 16S rDNA as *Brevibacterium* sp. *Bacillus* sp., *Kocuria* sp., *Streptomyces* sp. *Pseudomonas* sp. *Xanthomonas* sp. and *Micrococcus* sp.; and 15 isolates of fungi identified using ITS as *Alternaria* sp., *Cladosporium* sp., *Curvularia* sp., *Epicoccum* sp., *Fusarium* sp., and *Pseudotaeniolina* sp., which reflect a high diverse microbial community inhabit such harsh dry conditions. The current study discovered the presence of rock inhibiting fungi (RIF) known as *Pseudotaeniolina globosa* that is known as a bio-deteriorate agent in several cultural heritage sites around the world.

THE ROLE OF SIGNAL TRANSDUCTION IN REGULATION OF GENE EXPRESSION OF DROUGHT TOLERANCE IN WHEAT PLANT

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ABSTRACT

A Differential Display Polymerase Chain Reaction (DD-PCR) technique was selected for gene expression profiling to detect the up and down regulation of gene expression in shoots and roots of two cultivars Sahel1(tolerant) and Gammeiza7 (sensitive) in response to drought in wheat bread (*Triticum aestivum*). The twenty differentially expressed ESTs were obtained from shoots and roots. The sequences of ESTs were identified by using bioinformatics analysis. seven of which were shoots and 13 were for roots. Some of the results showed significant homology to predicted proteins were *TaAP2* with important roles in directing changes in gene expression during stress, Glycosyltransferases (GTs) catalyzed the transfer of sugar moieties to a wide range of acceptor molecules, Nelumbo nucifera protein NEN-4 played a role in cellular protection, restoration of plant functions under stress and exhibits protein-repair activity after heating to100°C, fructan 1-exohydrolase was important for regrowth of leaf tissue after defoliation, Ageratina adenophora ICE1 protein activated many downstream genes that confer chilling and freezing tolerance to plants, RNA polymerase II gene regulated of transcription, the synthesis of RNA from a DNA template, is one of the most important steps in control of cell growth and differentiation, serine/threonine-protein kinase maintained unperturbed photosynthesis inducing an oxidative stress response and playing a role in

salt stress signaling, NRC-1 was highly responsive to its environment and provided insights into some of the specific responses at the level of gene expression and prolamine genes stable proteins accumulated at massive levels due to the high level expression from extensively duplicated genes in endoreduplicated cells. Elucidation of the functions of genes could reveal the role of signal transduction in response to the mechanisms underlying drought tolerance in wheat cultivars to improve their yield&quality

GENETIC DIVERSITY, VARIETY IDENTIFICATION AND GENE DETECTION IN SOME EGYPTIAN GRAPE VARIETIES BY SSR AND SCoT MARKERS

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ABSTRACT

Leaves of fourteen grapes belong to seven Egyptian varieties with different morphological traits: Early Superior, Thompson, Flame, Red Globe, SO4, Ramsey and Dog Ridge were studied using twenty four SCoT primers and Seven SSR primers. Eight SCoT PCR bands showing pattern variation among grape varieties were recovered, cloned and sequenced. SCoT primers generated 362 PCR patterns with a total level of polymorphism of 77.1% and an average PIC of 0.044. The genetic similarity and relationships were estimated among the seven varieties according to Dice coefficient. SCoT analysis was effective in differentiating rootstock varieties (SO4, Ramsey and Dog Ridge) from other table varieties, in addition to green fruit (Early superior and Thompson) from red fruit varieties. SCoT was successful in characterizing all tested grape varieties by a total number of 73 unique positive and negative markers. Variety Dog Ridge was identified by the highest positive SCoT markers (7) .SSR PCR primers produced 73 fragments with a total amplicons polymorphism of 86.3% and an average PIC of 0.14. SSR analysis was effective in differentiating rootstocks varieties from other studied varieties. SSR technique was successful in characterizing all tested grape varieties by a total number of 19 unique positive and negative markers. SO4 was identified by the highest number of positive unique markers (5). Four SCoT sequenced bands: $SCoT3_{600}$,

$SCoT4_{450}$, $SCoT6_{200}$ and $SCoT12_{550}$ gave high sequence similarities, coverage and were close to potential genes located on grape genome or highly expressed in its transcriptome.

INITIAL CLUSTERING AND MOLECULAR IDENTIFICATION OF SOME EGYPTIAN BIOCONTROL ISOLATES

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ABSTRACT

Bacterial species are the most ubiquitous microorganisms that can be found in the Egyptian soil. Ten samples were collected from different geographical location through Egypt and isolated. These isolates showed different cluster of response toward our ecosystem, one group showed biocontrol activities in two directions, one when tested against Lepidopteron insects and nematodes in the fields and second against soil-borne disease, the other group showed plant-promoting growth to the plant through nitrogen fixation and accessibility to the minerals in the soil. It has been identified to the group level using phenotypic and biochemical tests. DNA isolation, PCR amplification and partial sequence analysis of 16SrRNA were conducted. The isolates were aligned using (ClustalW algorithm), compared with reference strain of *Pseudomonas fluorescence Pf5* genome sequence from NCBI Genbank database. BLAST comparisons of these 16SrRNA partial sequence identified two sets of 4 isolates are from Genus *Pseudomonads* and *Bacilli*, respectively and 2 of them are from Genus *Serratia*. Phylogenetic analysis was conducted by MEGA7 program using neighbor-joining method with 1000 replicates for bootstrap analysis. The phyologenetic tree analysis indicated that these isolates all shared some of the most common genes known for producing insecticidal toxins, proteases, putative hemolysins, hydrogen cyanide and novel secondary metabolites to infect and kill insects.

This paper is just the beginning of the initial clustering of some of these Egyptian isolates that contribute to both biocontrol and plant-promoting growth using comparative genomic analysis based on their virulence factors.