Name of Candidate: Abdullah Mohammed Ghaleb dawlah **Degree:** M.Sc.
Title of Thesis: Isolation and Molecular Characterization of the Trehalose Gene From Yemen Soil Bacteria Genome.
Supervisors: Dr. Mohammed Hassanin Soliman
Dr. Abdel-Kader Yossif Gamal El-Din
Department: Genetics **Approval: / / 2011**

ABSTRACT

Twelve bacterial isolates were purified from soil samples collected from three governorates in Yemen state. (Thamar, Hodidah and Sana'a). The trehalose gene is found in a wide range of organisms such as bacterial, yeast, fungi, plants, and animal's kingdoms .Unique biochemical and physical properties making the nonreducing disaccharide, trehalose a good protectant against various stresses (temperature and pH).

Ten random primers were used in the present study to characterize these bacterial isolates. Among them six primers generated reproducible and easily scorable RAPD profiles. The number of amplified DNA fragment was ranging 8 to 11 amplicons per primer The results of molecular analysis (RAPD) revealed 85.18% polymorphism and the similarity indices ranged from 44.1% to 97.1%.

Two specific oligonucleotide primers were used for the amplification of the two trehalose -6-phosphate synthesis (TPS or *Ost A* gene) and trehalose -6-phosphate phosphate (TPP or *Ost B* gene). The electrophorized band that is equivalent to 1425 bp in length was characterized in two bacterial strains (s1-A) and (s1-B). Moreover, DNA sequencing was performed to the retrieved band (1425 bp) .The reading frame of the 398 bp as a forward and 404 bp as a reverse reading frame was carried out in Germany for the proposed trehalose gene.

Finally, DNA sequencing alignment was screened using database of the trehalose gene in the gene bank. The 26 strrains of *E.coli* showed 100% and another 5 and 25 strains of *E.coli* showed 99% and 98% similarity respectively to the trehalose gene characterized in the present study.

Key words: *Trehalose gene*, *RAPD* , stress, *E. coli* ,Yemen

Name of Candidate: Mahmoud Abd El-Raheem Bassry **Degree:** M.Sc.
Title of Thesis: Genetic Transformation of Some Cereal Plants Targeting Abiotic Stress
Supervisors: Dr. Ebtissam H.A. Hussein
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Department: Genetics **Approval: / / 2011**

ABSTRACT

Environmental stresses, such as drought, increased salinity of soil, and extreme temperature, are major factors limiting maize productivity. In the present study, improving drought stress of three maize lines (Gz 649, Gz 639 and A188) was attempted. Immature embryo derived calli of the maize genotypes were transformed by *Agrobacterium*-mediated transformation and particle bombardment with the plasmid pSHX004 containing the *NPK1* gene for abiotic stress and the *bar* gene as a selectable marker. Nine independent transgenic events from the two transformation systems were obtained. The transformation efficiency of the *Agrobacterium*-mediated transformation was 5.2, 3.6 and 2.7% for the genotypes A188, Gz 639 and Gz 649, respectively. While, the transformation efficiency for the particle bombardment was 3.5% for Gz 649 and 10.7% for A188. Putative transgenic events have been tested by herbicide application through leaf painting, which showed tolerance to the herbicide Basta in comparison to non transgenics which showed wilting at the painted area. For molecular confirmation of putative transgenic plants, PCR analysis has been carried out and revealed the presence of both of the *NPK1* and the *bar* genes in the DNA of the putatively transgenic plants. Southern blot hybridization confirmed the integration of the gene of interest (*NPK1*) into the genome of the maize transgenic plants. The copy number of the *NPK1* transgene introduced into maize by *Agrobacterium* ranged between 1 to 5 copies. While, the copy number of the *NPK1* transgene integrated into maize by particle bombardment was 10 copies. These results indicated that the *Agrobacterium*-derived maize transformants revealed lower transformation frequency and transgene copies than their counterparts obtained by particle bombardment. Under water deficit conditions transgenic plants maintained a higher growth and showed increased tolerance to stress conditions compared to non-transgenic plants. These results demonstrate that the *NPK1* gene might play a role in the protection of plants under water deficiency-stress conditions.

Key words: Maize (*Zea mays* L.)**,** *NPK1*gene, Abiotic stress, *Agrobacterium* and Biolistic gene gun.

Name of Candidate: Mohamed Ahmed Ezz Alregal **Degree:** M.Sc.
Title of Thesis: Molecular Study on Environmental Stress (Heat Shock-Genes)
Supervisors: Dr. Mohamed Hassanin Soliman
Dr. Abd Alkader-Youssuf Gamal El-Din
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Department: Genetics **Approval:** 20/ 9 /2011

ABSTRACT

Degenerate primers are particularly useful in amplifying homologous genes from different species. The present study aimed to investigate the importance of degenerate primers and the HSP70 family signature to create a new specific motif for HSP70 proteins family and Described a method for designing degenerate primers for a given multiple alignment of DNA sequences of HSP70 gene family using Clustalw algorithm.

An *Insilco* approach was used to find a homology between more than one Accession numbers of DNA sequences, (X67711.2) was for *Oryza sativa* (HSP70), (AY372071.1) was for *Nicotiana tabacum* (HSP70) and (L41253.2) was for *Lycopersicon esculentum* (Hsc70), the three accession numbers were retrieved by the BLASTn program depend on their expected value (E-value).

Multiple sequence alignment was performed by clustalw algorithm to produce a conserved blocks and determined the consensus region was used to produce the forward and reverse primer by the primer select module of DNA STAR LASER GENE 7.0. An *Insilico* PCR module of FASTPCR program ver.4.0.8 was performed to detect the melting temperatures (Tm) and Predicted the PCR product size. The results of deigned degenerate primer showed that there was a homology found between the designed primers and the DNA templates for the previous three accession numbers with at least 80% identity. The result of degenerate PCR showed that the three bands of the amplified PCR products of the three accession numbers were detected at the same molecular weight of (385bp) with a difference about 15 bp compared to the *Insilco* PCR product (385 bp). Degenerate PCR was used to isolate the consensus coding sequence of HSP70 gene family for *Nigella sativa* and sequenced the predicted band. The results of degenerate PCR showed that the amplified PCR products for the three accession numbers and *Niegella sativa* PCR products were detected at the same molecular weight (٣٨٥ bp) and the result of *Nigella sativa*, sequenced band (385bp) was justified and corrected to be 345bp showed a homology to HSP70 gene family and recorded with a new accession number (HM803244) in the NCBI. The bioinformatics data were retrieved from the curated databases of protein to detect the conserved sequences among the records of HSP70. A new motif was built and all the statistical analysis of the new motif was processed to build the dendogram based on the physiochemical properties of amino acid sequences. A new specific motif was designed by PRATT tool which depended on the multiple sequence alignment algorithm and by using position specific iterative BLAST (PSI-BLAST). The new specific motif was used to construct the polygenetic tree from multiple sequence alignment for every taxonomy of (bacteria, viridiplantae and metazoa) and the single peptide motif was converted to predict the 3-D structure. The 3-D structure templates in the PDB (Protein Data Bank) using (scanprosite) database search. The result of the predicted 3-D motif showed a highly similarity and stabilization to the other crystallography templates of HSP70 in different organisms.

Key words: Degenerate primers, *Insilico* PCR, 3-D motif, Clustalw

Name of Candidate: Ibrahim Osamy Ibrahim Hassan **Degree:** M.Sc.
Title of Thesis: Biotechnological Studies on the Irradiated Potato (*Solanum tuberosum*) with Gamma Rays
Supervisors: Dr. Mohamed Hassanin Soliman
Dr. Abdel-Kader Gamal El-Din
Dr. Abdel-Shafy Ibrahim Ragab
Department: Genetics **Approval:** 19/10/2011

ABSTRACT

Bacterial wilt or brown rot disease caused by *Ralstonia solanacearum* causes extensive annual losses of different crops especially potato crop. It is considered as one of the limiting factors for potato production and exportation in Egypt. Therefore, the main purposes of this study were to investigate the effect of gamma rays on two potato cultivars (Diamant and Spunta). And, to obtain new genotypes of potato resistant to bacterial wilt disease. This study was carried out in the field and Biotechnology laboratory of the Plant Res. Dept., Nuclear Res. Center, Inshas, Egypt and Genetics Dept., Fac. Agric., Cairo Univ., during 2008-2011. In the field experiment, dry tubers of potato cultivars were irradiated by different doses of gamma rays (20, 30 and 40 Gy) to study the effect of gamma rays on the vegetative and yield traits. The results showed that there are no significant differences between cultivars for all studied traits except a number of tubers per plant trait. Also, there are only highly significant and significant differences between treatments for weight of tubers per plant and number of tubers per plant traits, respectively. However, there are only significant differences between the interactions of cultivars and treatments for plant height and weight of tubers per plant traits. Six genotypes were selected from M_1V_2 generation depending on high yield for RAPD analysis to determine their genetic variability from its parents at molecular level using 11 primers. The results of RAPD analysis showed that 11 primers generated 56 distinct bands of which 31 (55.4%) were considered as polymorphic. The similarity indices of six genotypes of potato and its parents ranged from 70 to 91%. The highest genetic similarity 91% was found between D20 genotype and D0 (Diamant control), on the other hand, the lowest genetic similarity 70% was found between S30, S40 genotypes and its parent S0 (Spunta control). In the artificial infection experiment under *in vitro* condition, the irradiated and non-irradiated plantlets of potato were cultured on medium inoculated with local virulent isolate of *R. solanacearum.* The results showed that all *in vitro* plantlets of the treatments in Diamant and Spunta cultivars were susceptible except S20 treatment was resistant to the infection with *R. solanacearum.* Protein analysis showed that S20 genotype (resistant mutant) displayed 2 negative unique bands that may be responsible for resistance to *R. solanacearum.*

Key words: Potato, gamma rays, mutation, brown rot, RAPD

Name of Candidate: Amr Said Mohamed **Degree:** M.Sc.
Title of Thesis: Genetical And Biochemical Evaluation Of Some Woody Trees Genetic Resources
Supervisors: Dr. Mohamed Hassanen Soliman
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Department: Genetics **Approval:** / /

ABSTRACT

Genetic polymorphism was investigated in six conifers representing four Pinus species, *i.e.* (*P.halepensis, P.canariensis, P.pinea, and P.roxburghii*) which belong to family *Pinaceae* and two members of family *Taxodiaceae*, i.e. (*Sequoia sempervirens and Taxodium distichum*). In this respect, biochemical (proteins and isozymes), as well as molecular (RAPDs and ISSRs) analysis were investigated. Proteins and peroxidase banding patterns resulted in extensive polymorphism among conifers under investigation, however, Adh isozyme banding patterns were not informative in this concern. RAPD analysis exhibited a total of 66 bands, out of them 25 bands were polymorphic (37.88%). Five ISSR primers generated reproducible and informative amplified products, those were used to distinguish between the six conifers, Thirty eight bands were polymorphic out of a total of 81 bands with 47.95% polymorphism which can be considered as useful markers for identifying conifers. Based on combined data obtained by proteins, peroxidase, RAPD and ISSR analysis, it was possible to discriminate between the six conifer trees under investigation. The present study indicates that the application of biochemical and molecular fingerprinting of the six conifers provided a solid ground that will allow an easier and faster genetic identification of other woody trees species

Keywords: Conifers, *Pinus*, *Sequoia*, *Taxodium*, RAPD, ISSR, SDS-PAGE, Peroxidase, Alcohol dehydrogenase.