Agricultural Research & Technology
Open Access Journal
ISSN: 2471-6774

Juniper Publishers
key to the Researchers

Research Article
Volume 28 Issue 1 - November 2023
DOI: 10.19080/ARTOAJ.2023.28.556393

Agri Res & Tech: Open Access J

Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels

Noha M E M Hammad[1]*, Moustafa M Eldakak[1], Mounir Moussa[1], Hemaid I A Soliman[2] and Mohamed M Yacout[1]

1Department of Genetics, Faculty of Agriculture (El Shatby) Alexandria University, Alexandria, Egypt

2Plant Genetic Resources Department, Desert Research Center, El-Matariya, Cairo, Egypt

Submission: October 26, 2023; **Published:** November 30, 2023

***Corresponding author:** Noha M E M Hammad Department of Genetics, Faculty of Agriculture (El Shatby) Alexandria University, Alexandria, Plant Genetic Resources Department, Desert Research Center, El-Matariya, Cairo, Egypt

Abstract

Salvadora persica L. is a highly threatened halophyte plant that can endure extreme desert environments, it has many Medicinal and economical potentials. S. prescica is a promising organism for the identification and characterization of mechanisms related to abiotic stress tolerance. Abiotic stresses usually cause protein dysfunction so maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under stress. Heat-shock proteins 90 (Hsp90) co-chaperones are constitutively expressed in most organisms. The major role of Hsp90 involves protein-folding assembly in the cell, as well as plant growth and response to environmental stimuli. It also plays a key role in signal-transduction networks, cell-cycle control, protein degradation and protein trafficking. This study aims to identify the Partial sequence of the Hsp90 gene of Salvadora presica for the first time and spot its adaptive mechanisms to salinity up to 750mM (hyper salinity) by screening on the molecular basis of Hsp90 gene expression by (RT- qPCR) analytical technique compared to proline levels (biochemical aspect) a very important metabolite for salinity tolerance.

Keywords: Halophyte; Salinity tolerance; Salvadora presica; Chaperone; Proline; HSP

Abbreviations: HSP: Heat Shock Protein; PCR: Polymerase Chain Reaction; RT-qPCR: Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction

Introduction

Climate change and heat-related temperature increases will bring about rising sea levels and increasing drought, both of which will contribute to increasing salinization in many regions of the world. Many crops may suffer as a result [1]. Salinization of soil is one of the most serious threats to irrigated crop production in arid and semi-arid regions. Salinity is a soil condition characterized by a high concentration of soluble salts. Soils are categorized as saline when the EC is 4 dS m-1 or more which is equivalent to approximately 40 mM NaCl and generates approximately an osmotic pressure of 0.2 MPa. Halophytes are plants that thrive in salty environments. A high concentration of soluble salts in the substrate allows halophytic species, which make up 1% of the world's flora, to thrive and complete their life cycle. Halophytes can flourish in a few habitats, including salt marshes and coastal areas. Salinity levels, which are harmful to many crop species, are advantageous for halophytic species. Both innate and learned defensive mechanisms can be used to respond to environmental stress. Salt avoidance and salt tolerance are the two primary categories into which the mechanisms of salt resistance in halophytes are typically divided. The technique behind salt tolerance may involve specific physiological and/or biochemical changes in the plants that allow the plant to preserve protoplasm viability during salt ion accumulation inside the cells [2].

Salvadora persica L. popularly known as the Toothbrush tree, Saltbush tree and Meswak, is a highly threatened medicinally, economically important, slow- growing xerophytic medium-sized tree or shrub with crooked trunk and drooping branches [3] belonging to the little-known family *Salvadoraceae* facultative halophytic desert species that can endure extremely high salinity levels. Alkaloids, tannins, saponins, and sterols are just a few of

the bioactive substances found in *S. persica* plant that are used in both the food and cosmetic industries, it can grow in a variety of habitats, including deserts, heavy soil, non-saline soil, marshy soil, and dry regions with little water. Many species need fresh water to germinate, but *S. persica* can do so in saline water [4].

Scientific classification according to [5].

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Brassicales

Family: Salvadoraceae

Genus: Salvadora

Species: persica

Heat-shock proteins (HSPs)/chaperones are the molecules involved in cellular homeostasis that are known to be essential for protecting plants from stressors. Stress usually causes protein dysfunction. Maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under stress, through membrane protein folding, assembly, translocation, degradation not only under stress but also in many normal cellular processes, and stabilization of proteins and membranes, and can assist in protein refolding, restore protein structure., under stress conditions. They can play a crucial role in protecting plants against stress by re-establishing normal protein conformation [6].

Heat shock protein 90 (HSP90), is one of the heat shock families, co-chaperones, which are expressed in eukaryotes, cytosol, chloroplasts, mitochondria, and endoplasmic reticulum, the most abundant cytosolic proteins in eukaryotes, amounting to ~1% of soluble protein even in the absence of stress [7]. HSP90 is distinct from many other molecular chaperones in that most of its known substrates to date are signal transduction proteins [6]. HSP90 protein controls how plants react to stimuli like heat, cold, salt, heavy metals, phytohormones, light/dark transitions, and drought. In addition, HSP90 protein helps other proteins with appropriate protein folding, signaling networks, cell cycle regulation, protein degradation, and protein trafficking. There is, however, little data on HSP90 protein in plants under salinity stress, and most of the previous research concentrates on glycophytes, such as rice, maize, soybean, potato, and beans [8]. DNA sequencing techniques are key tools in many fields. Many different sciences are receiving the benefits of these techniques, ranging from archaeology, anthropology, genetics, biotechnology, molecular biology, and forensic sciences, among others [9]. DNA sequencing technologies evolved through three generations, Sanger represent the first generation of sequencing, then massively parallel the Second generation of sequencing finally real time and single molecule represent examples of the third generation [10]. Reverse transcription followed by quantitative polymerase chain reaction analysis, or qRT-PCR, is an extremely sensitive, cost-effective method for quantifying gene transcripts from plant cells. The availability of nonspecific double-stranded DNA (dsDNA) binding fluorophores, such as SYBR Green, and 384-well-plate real-time PCR machines that can measure fluorescence at the end of each PCR cycle make it possible to perform qRT-PCR on hundreds of genes or treatments in parallel. This has facilitated the comparative analysis of all members of large gene families, such as transcription factor genes [11]. RT-qPCR is the gold standard for gene expression research. However, for this claim to be valid, RT-qPCR studies must test and optimize the quality of its RNA templates and assays [12]. Therefore, this study aims to detect the partial sequence of HSP90 gene, and its expression levels using quantitative polymerase chain reaction (qRT-PCR) in *Salvadora presica*, exposed to various extreme salinity levels up to 750 mM NaCl.

Materials and Methods

A. Plant materials. *Salvadora presica* seedlings are rare. We succeeded in collecting a limited number of them from two local sellers in Giza, Alexandria and from the desert research center.

B. DNA ISOLATION. *S. persica* is notoriously recalcitrant to many common DNA extraction methods, genomic DNA was isolated from young leaves by using the modified CTAB method described by [13].

C. PCR Amplification of Hsp90 gene

i. Cycling /amplification of Hsp90 via Degenerate primers: Degenerate PCR primers used in this study were described by [14]. And were constructed based on an amino acid alignment. Forward primer Hsp90 IF was designed based on the conserved region DALDKIR. The two reverse primers HSP90IIIR and HSP90IVR were based on MKAQALR and MEEVD respectively, the latter of which marks the C terminal of *HSP90* proteins. Experiment A amplification was carried out in two rounds:

Round 1: Initial PCR cycling (Using Hsp90 IF and HSP90IVR primes). The polymerase chain reaction was applied in 20µl final reaction and consisted of 10 µl master mix (2X TOPsimple™ DyeMix-nTaq); 1 µl (10 pmole) of primer (LIGO) and 50ng of genomic DNA. DNA amplification was performed in a Biometra T1 gradient thermal cycler for 35 cycles, with initial denaturation for 2 min at 94°C. Each cycle consisted of denaturation at 95°C for 1 min.; annealing at 65°C for 1.30 min (20 cycles of 35) and the remaining (15 cycles) annealing at 55°C for 1.30 min; each cycle extension at 72°C for 3 min and final extension at 72°C for 2 min.

Round 2: Semi-nested PCR re-amplification. The products of the initial PCR previously mentioned (selected based on the lowest yielded bands number in the initial PCR) amplified using the same forward Hsp90 IF and HSP90IIIR ,1ul of initial PCR product as a template, primers quantities and mater mix remain the same, the reaction was performed using the same thermal cycler for 35

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

cycles after initial denaturation for 2 min at 94°C. Each cycle consisted of denaturation at 95°C for 1 min; annealing at 55°C for 1.30 min; extension at 72°C for 2 min and final extension at 72°C for 2 min. The combinations of forward primers and PCR products were fractionated on an agarose gel to check for specificity and to monitor for contamination using a negative control, and Enzynomics 100 bp DNA Ladder Marker was used. Agarose gel 1.5% agarose (Puregene agarose le quick dissolve) was run for 40 min (100v) in 1xTBE buffer the gel was stained by ethidium bromide then visualized in UV light and photographed.

ii. PCR Products Sequencing

PCR amplifications were performed on a thermal cycler (Peq Primus 25). PCR amplicon resulted from round two by degenerate primers forward Hsp90 IF and reverse HSP90IIIR was bidirectional sequenced. all amplicons were sequenced using sequencing primers same as primers used in PCR protocol which are shown in PCR second reaction and the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) on an ABI 3730 capillary sequencer following manufacturer's instructions.

iii. Sequence Analysis

A uniform search for the sequence database was carried out for retrieving sequences at websites to find and compare Hsp90 gene among different species. It Aligned and compared with related sequences from the nucleotide database (blastn and blastp) comparing Hsp90 gene orthologs across different species. by using the Basic Local Alignment Search Tool of the National Centre for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov. and EBI-EMBL FASTA PROTEIN, Tools > Sequence Similarity Searching > FASTA (blast)respectively. Also to Ncbi Orf finder, Ncbi Conserved domain and phylogeny.

D. Sequence Expression Assessment (RT-PCR)

The expression of theHsp90 gene was performed using real-time polymerase chain reaction (RT-PCR).

1. RNA Isolation. isolation of total RNA from leaves (100 mg) of control and from which exposed to salt stress treatments according to an RNA extraction kit (Plant Total RNA Mini Kit, Geneaid). Stress treatments were done on seedlings irrigated with 100 mM NaCl,250 mM NaCl, 500 mM NaCl and 750 mM NaCl for 10 days.

2. Real-time quantitative RT-PCR (qRT-PCR) analysis. The transcript levels of the Hsp90.1 gene were analyzed using quantitative real-time RT-PCR in a Real-time PCR machine (Rotor gene q, quiagen, Germany). The qRT-PCR was performed using one microgram of total RNA, SYBR green with high ROX, (enzymic kit, Korea). SYBR green participate with cDNA and specific primer of HspI-F and HspI-R of *S.presica* Hsp 90 and reacted together, real-time PCR process was performed using the following procedure: reverse transcription at 50°C for 2 min initial denaturation at 95 °C for10 min, then each cycle at 94 °C for 15 s, annealing and extension at 52°C for 60s. The 18s gene is the internal reference gene. Five independent sets of experiments were performed with three technical replicates each to finalize the data. and delta-CT value (the difference between CT values obtained for the gene of interest and normalizer or housekeeping gene), and the difference between the threshold cycle (CT) values obtained for the HSP90 and housekeeping gene 18S rRNA was calculated, samples were analyzed, and the average of Ct values were calculated. To reveal the absence of contamination or primer dimmers, a non-template control (NTC) reaction with each primer pair was run. The delta-delta-CT (2−ΔΔct) equation [15]. Was used to compare the expression of the Hsp90 gene between treatments and controls in terms of fold difference [16]. The mean of standard deviations of Log2 fold change (log2FC) was used to rank the performance of the gene.

E. Biochemical analysis (Proline content assay)

Proline content was determined according to the method of [17]. Estimated through photometric analysis of 5 treatments respectively, Control,100mM, 250mM, 500mM and 750mM, absorbance was measured at 520 nm by GENESYS 10S UV–VIS spectrophotometer (Thermo Fisher Scientific, Medison, USA). using toluene for a blank.

Results and Discussion

A. Extracted genomic DNA of *Salvadora presica*

DNA isolation of *S. persica* wasn't an easy process due to the high glycosylate level that hindered the isolation of its DNA with a sufficient quantity and high purity. We tried several methods, the manual method described by [13]. from leaves was efficient (Figure 1).

B. Hsp90 gene amplification

We tried to get an Hsp90 gene sequence in a cheap easy way depending on the conserved region within the Salvadora presica genome a very promising salinity-tolerant plant with limited genetic information.

B.1. First PCR reaction (round1)

The degenerate primer of Hsp90 from [15]. Was used in the phylogenetic analysis of eukaryotes. Using degenerate PCR products for direct sequencing usually has low probabilities. However, we thought about spotting this and investigating it more. First, we used a 2-step PCR, tried to increase the stringency of primers, selected and minimized bands numbers as possible with the same methods of minimizing unspecific bands (several cycling conditions were tested but those described in materials and methods give the lowest number of bands) [18,19]. And choose lowest number of bands (ex; sample A) yielded in first PCR reaction step (Figure 2).

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

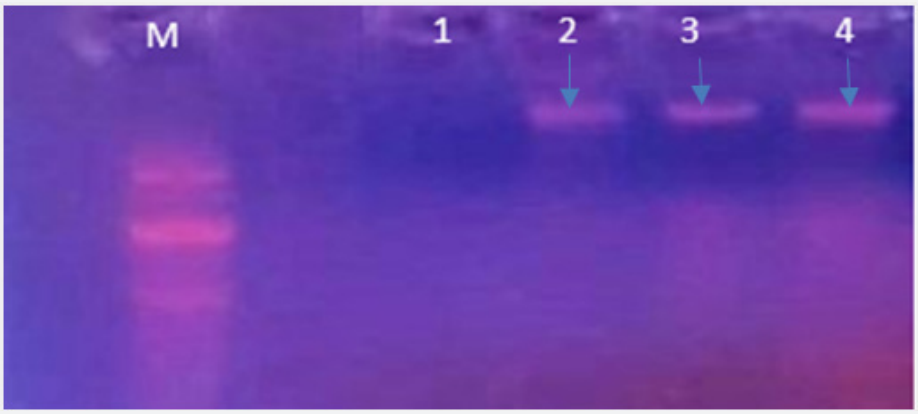

Figure 1: Agarose gel electrophoresis of extracted genomic DNA. Lane 1 = negative control; lane 2:3:4= Salvadora presica genomic DNA; M = ENZYNOMICS 100 bp DNA marker.

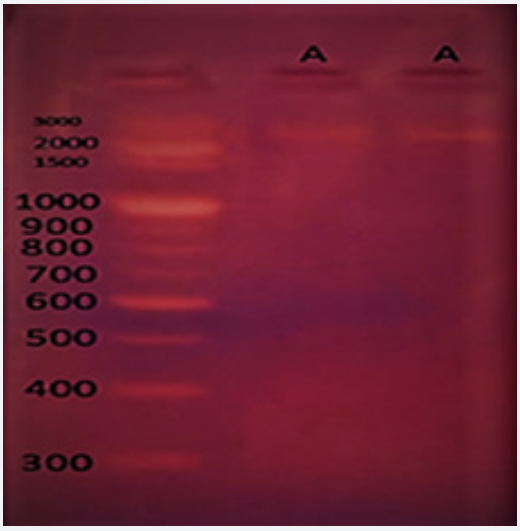

Figure 2: Separation of first round PCR products fractioned on agarose gel lane1 contains ENZYNOMICS 100 bp DNA marker lane 2 and 3.

B.2. Second PCR Reaction (round 2)

Then cycle the previous step product as a template for the second PCR reaction (round 2) with the other reverse primer, products yielded from the first and second PCR were separated on a gel, then a clear band yielded in round 2, hoping not to find that this band contains several products within the same size. Sample 2 was bi-directionally sequenced (Figure 3).

B.3. Sequencing

Sequences yielded a conserved region with meaningful alignment results with heat shock (TGCCAGGACTCAGTGGGACGTCATTCCCAGTGTCAAGCAGCATGGAGAGTCAGCTCCCCTATCAGACATGACCACTCTCTCCAGGTCAGCTCATAAGTTCCTTGACAGTCATGACCAGTCCCGAATGATTTCTTTTTCCCCCCCCTTTCTTTCTTTCCTCCTTTGATGAAACCTGCCAGTTCAGACCCTTGTGGCCAGCTGAACCGACTTTCTTTCAGACTCTCTCGTTCGGCTGGCCAAATACACACTCGTGGATGGCATCAAGAATAAGAAGGAATTCGCATTCTTTCTTTTTTCTCCCCGCAAGGGAGGGGTAATTACCGACAGCTTGCTTGCTCCAGCG)

C. **Hsp90 gene alignment**

Alignment gave a partial sequence with reasonable results at different algorithms and databases. The Basic Local Alignment Search Tool (BLAST) is a computer algorithm that is available for use online at the National Center for Biotechnology Information (NCBI) website, as well as many other sites. BLAST can rapidly align and compare DNA sequences with a database of sequences and finds regions of local similarity between sequences. The pro-

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

gram compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches (Figure 4). BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families. Which makes it a critical tool in ongoing genomic research. Tools like BLAST have enabled scientists to study the genetic blueprint of life across many species, and it has also helped connect biology and computer science in the maturing field of bioinformatics. BLASTn can be used to compare a nucleotide sequence with a nucleotide database [20].

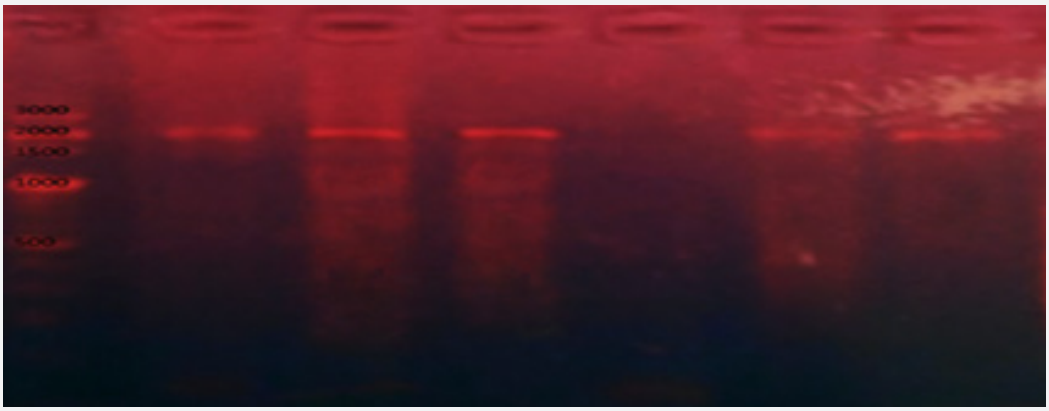

Figure 3: Separation of semi nested PCR (round 2) products fractioned on agarose gel lane1 contains ENZYNOMICS 100 bp DNA marker lane 1,2,3,5 and 6 contains products resulted from the lowest band yielded samples of cycle 1 as a template for them.

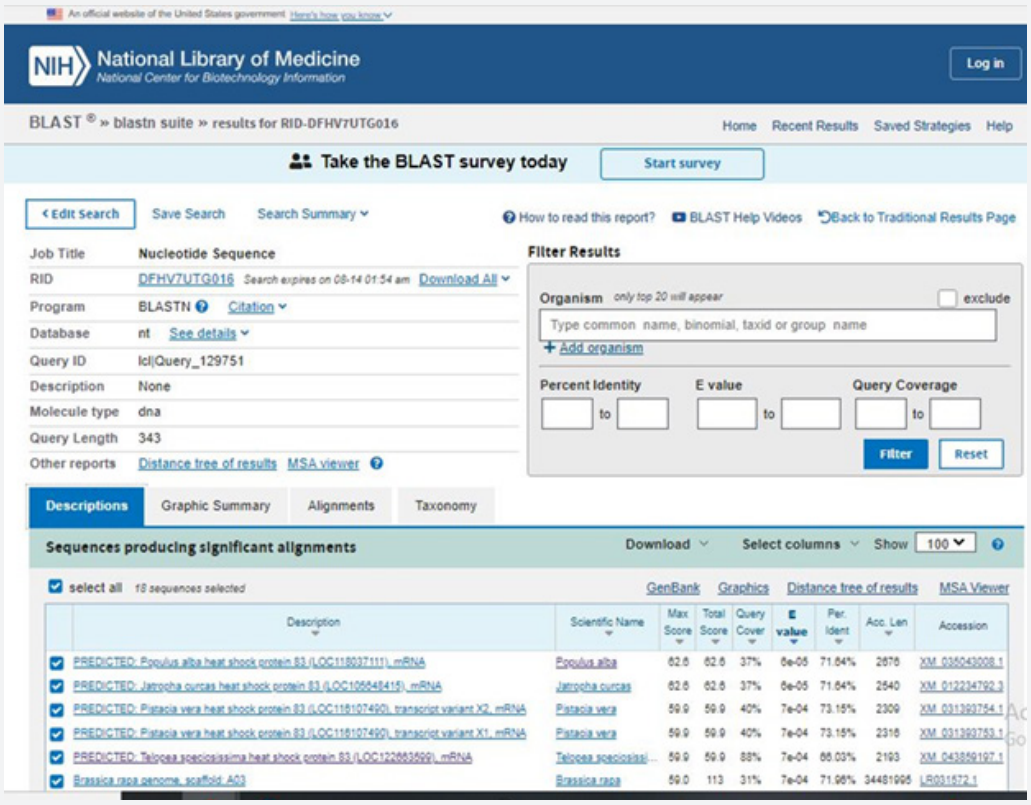

Figure 4: Alignment results via BLASTn (somewhat similar) program of national center for biotechnology (NCBI).

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

BLASTn (nucleotide blast) alignment program of the National Center for Biotechnology (NCBI).

It aligned with *Pistachia chinensis* mRNA and paunch of Hsp90 genes with a high percentage of identity in several plants (Populus alba, Jatropha (*curcas*, *Paver somniferm*, *Pistacia vera*). Also, one of the closest sequenced relative taxonomically (*Brassica spp* and *Salvadora pressica*) belonging to the same family *Brassicaceae* were detected as a hit of this alignment.

According to the phylogenetic tree constructed based on blastn alignment *pistachcia chinensis* heat shock protein (Hsp90) mRNA, complete cds was the closest, due to the available and detected data (Figure 5). Most of these plants are tolerant to various harsh environments [21-25].

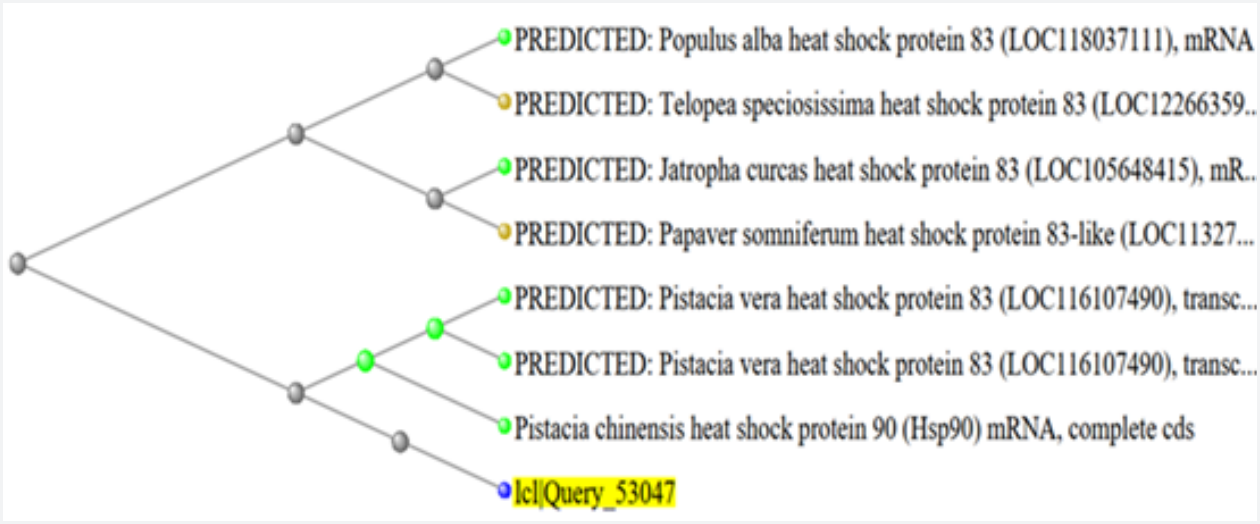

Figure 5: Distance tree constructed depending on blastn alignment results of (figure 4) of national center for biotechnology (NCBI).

TBLASTX

A tool compares all six reading frames of a query sequence to all six reading frames of a database intensive algorithmic feat that can bring even modern computers to a grinding halt if not used properly [16].

NCBI's Reference Sequence (RefSeq) database is a collection of taxonomically diverse, non-redundant and richly annotated sequences representing naturally occurring molecules of DNA, RNA, and protein. Included are sequences from plasmids, organelles, viruses, archaea, bacteria, and eukaryotes. Each RefSeq is constructed wholly from sequence data submitted to the International Nucleotide Sequence Database Collaboration (INSDC). Similar to a review article, a RefSeq is a synthesis of information integrated across multiple sources at a given time. RefSeqs provide a foundation for uniting sequence data with genetic and functional information. They are generated to provide reference standards for multiple purposes ranging from genome annotation to reporting locations of sequence variation in medical records. The RefSeq collection is available without restriction and can be retrieved in several different ways, such as by searching or by available links in NCBI resources, including PubMed, Nucleotide, Protein, Gene, and Map Viewer, searching with a sequence via BLAST, and downloading from the RefSeq FTP site [26].

When compared to the reference sequence- RNA database(transcript Reference Sequences, Molecule Type: cDNA) it showed a high query coverage percent of hsp90 gene in several plants (*Zingiber officinale, Panicum virgatum, Zea mays, Oryza sativa Japonica, Oryza glaberrima, Lolium perenne, Brassica napus, Oryza brachyantha, Brassica oleracea, Triticum urartu, Triticum aestivum, Hordeum vulgare, Prunus mume, Aegilops tauschii subs, Syzygium oleosum, Raphanus sativus, Arachis duranensis, Salvia splendens, Raphanus sativus , Solanum dulcamara , Solanum stenotomum , Solanum verrucosum , Solanum tuberosum , Citrus sinensi , Arabidopsis thaliana, Diospyros lotus, Gossypium raimondii, Erigeron Canadensis, Manihot esculenta ,Mangifera indica, Rosa chinensis , Juglans regia , Nicotiana sylvestris , Tarenaya hasslerian, Populus trichocarpa, Arachis duranensis, Andrographis paniculata, Erigeron canadensis , Manihot esculenta , and Gossypium hirsutum*) (Figure 6).

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

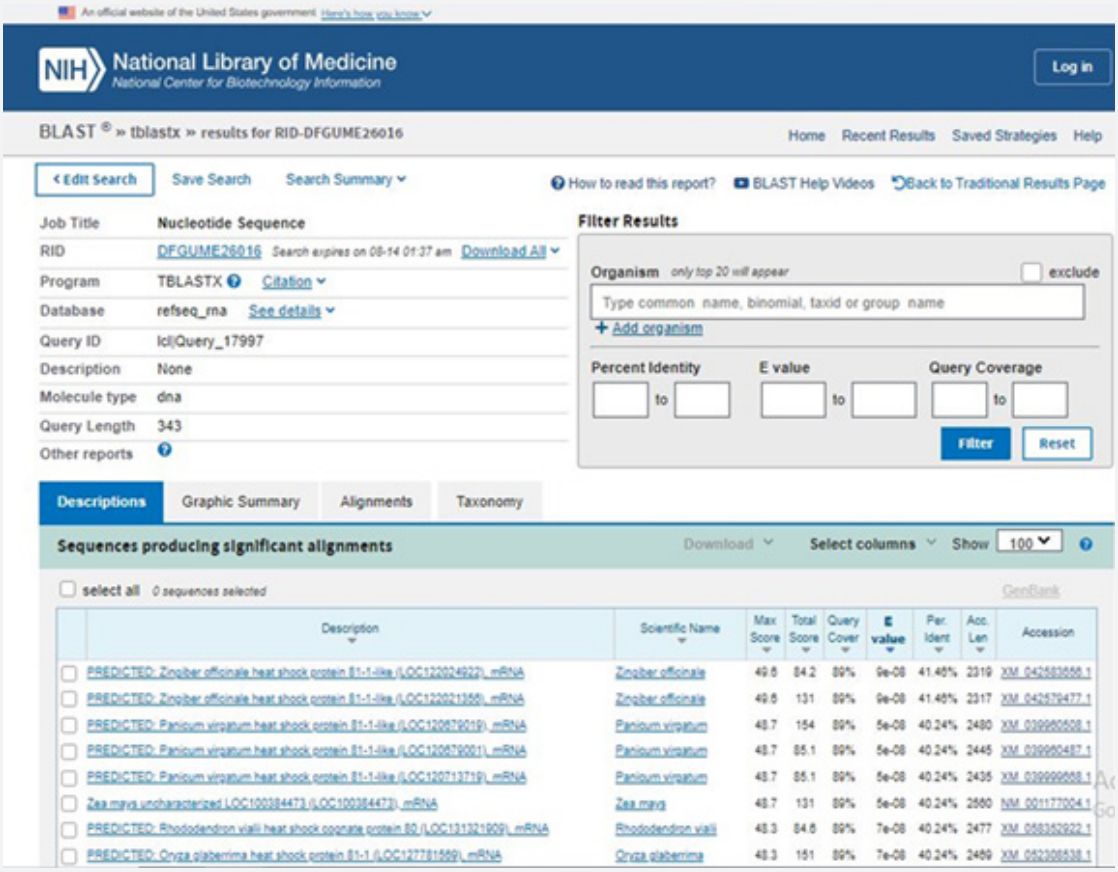

Figure 6: Alignment results based on TBLASTX program of national center for biotechnology (NCBI).

BLASTX can take a nucleotide sequence, translate it, and query it versus a protein database in one step [27].

It aligned with (*Oryza sativa, Arabidopsis thaliana, Nicotiana tabacum, Ipomoea nil, and Zea mays). Arabidopsis thaliana* is a *brassicals*, one of the taxonomically closest sequenced plants to *Salvadora presica* (Figure 7).

a. FASTA alignment

The FASTA package of sequence comparison programs have been expanded to include FASTX and FASTY which compare a DNA sequence to a protein sequence database, translating the DNA sequence in three frames and aligning the translated DNA sequence to each sequence in the protein database, allowing gaps and frameshifts [28].

FASTX

It aligned with (*Oryza sativa, Arabidopsis thaliana, Ipomoea nil, Nicotiana tabacum, and Zea mays*) (Figure 8).

FASTY

A tool compares a DNA sequence to a protein sequence database comparing the translated DNA sequence in forward and reverse frames. It aligned with *(Nicotiana tabacum-Ipomoea nil-Oryza sativa and Arabidopsis thaliana)* (Figure 9). We should consider that there are no data of heat shock sequences on Salvadora presica, which is distinct taxonomically, and have quite distance to the closest sequenced relative.

Hsp90 sequences they are large and very diverse and has several nomeculture [29]. Search was performed using nucleotide sequence corresponding to the Hsp90 gene in Salvadora presica in this study compare it and retrieve similar sequences from other plants. We blast it through NCBI nucleotide mega blast but it fails to recognize it [30]. However, blastn gives a reasonable result, also Ebi fast and blast x were successful. Homology (common ancestry and similar structure) can be reliably inferred from statistically significant similarity which is recognized from statistical estimates (E)-values, most investigators are more comfortable describing similarity in terms of "per cent identity", [31]. Statistically significant similarity can be determined in a BLAST and FASTA search, but to infer that two proteins are homologous does not guarantee that every part of one protein has a homolog in the other. BLAST and FASTA calculate local sequence align-

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

ments; local alignments identify the most similar region between two sequences. The most common reason homologs are missed is because DNA sequences, rather than protein sequences (or translated DNA sequences), are compared. Protein (and translated DNA) similarity searches are much more sensitive than DNA: DNA searches. DNA: DNA alignments have between 5-10-fold shorter evolutionary look-back time than protein: protein or translated DNA: protein alignments [29]. That's a clue why FastX, FastY and BlastX represent a translated sequence that gives a larger scale results number than Blastn. Results have varied as some parameters can be more precise or give better results than others depending on the used searches or algorithms or database as the following figures explain. can be due to several reasons Hsp90 complexity and sequences characteristics, as a longer sequence is more difficult to analyses [29]. Also, because the expectation value depends on database size, an alignment score found by searching 10,000, *-99000 entry database will be 100-fold+ less significant than the same score found in a search of a 100,000-entry database [29,32]. explained that BLASTP is a more selective algorithm that rarely calculates high scores for unrelated sequences, so that lower "raw" similarity scores would still have high statistical significance.

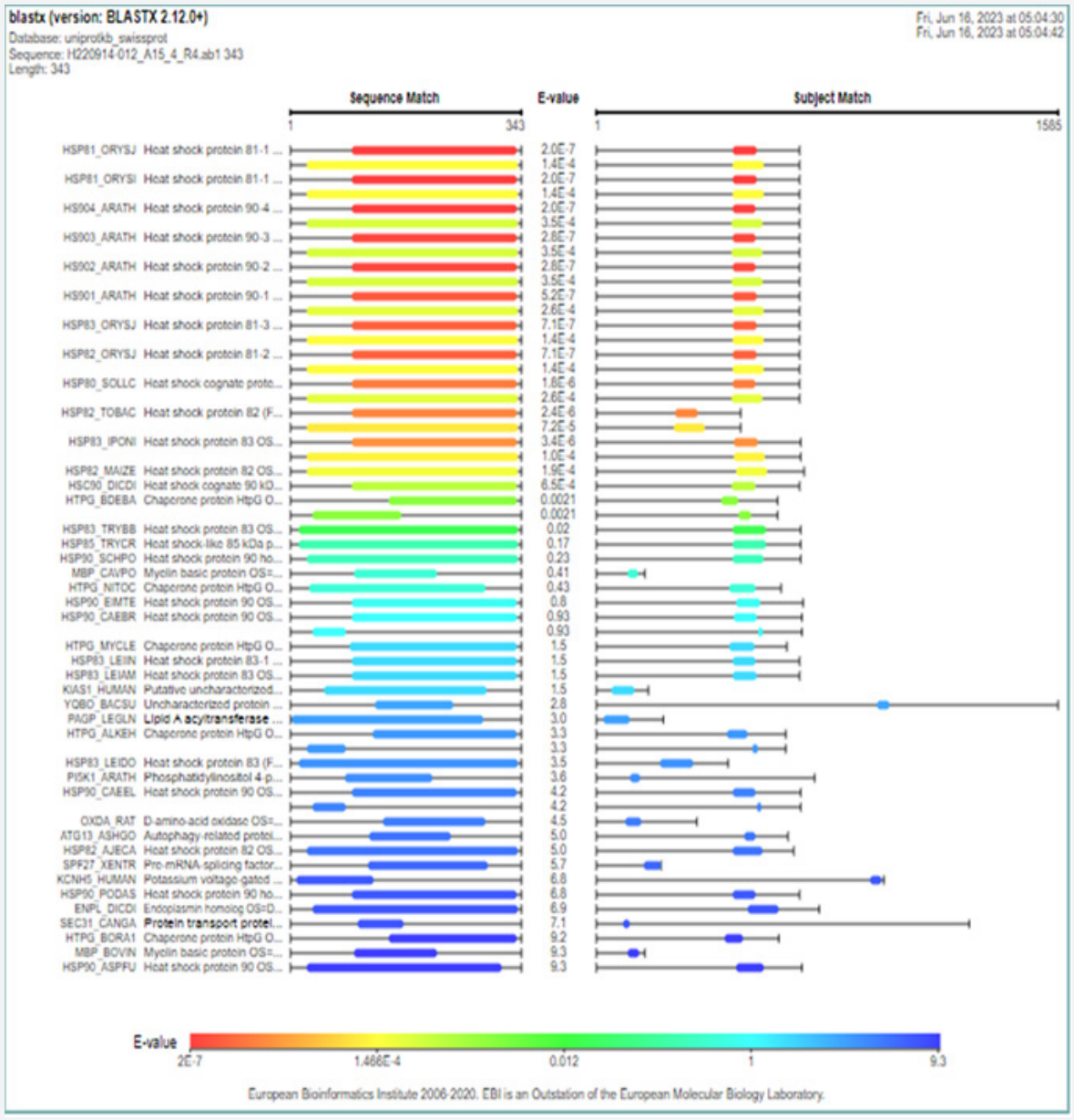

Figure 7: visual output of (NCBI BLAST+) alignment via protein similarity search of BLASTX for DNA Sequence through European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI).

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

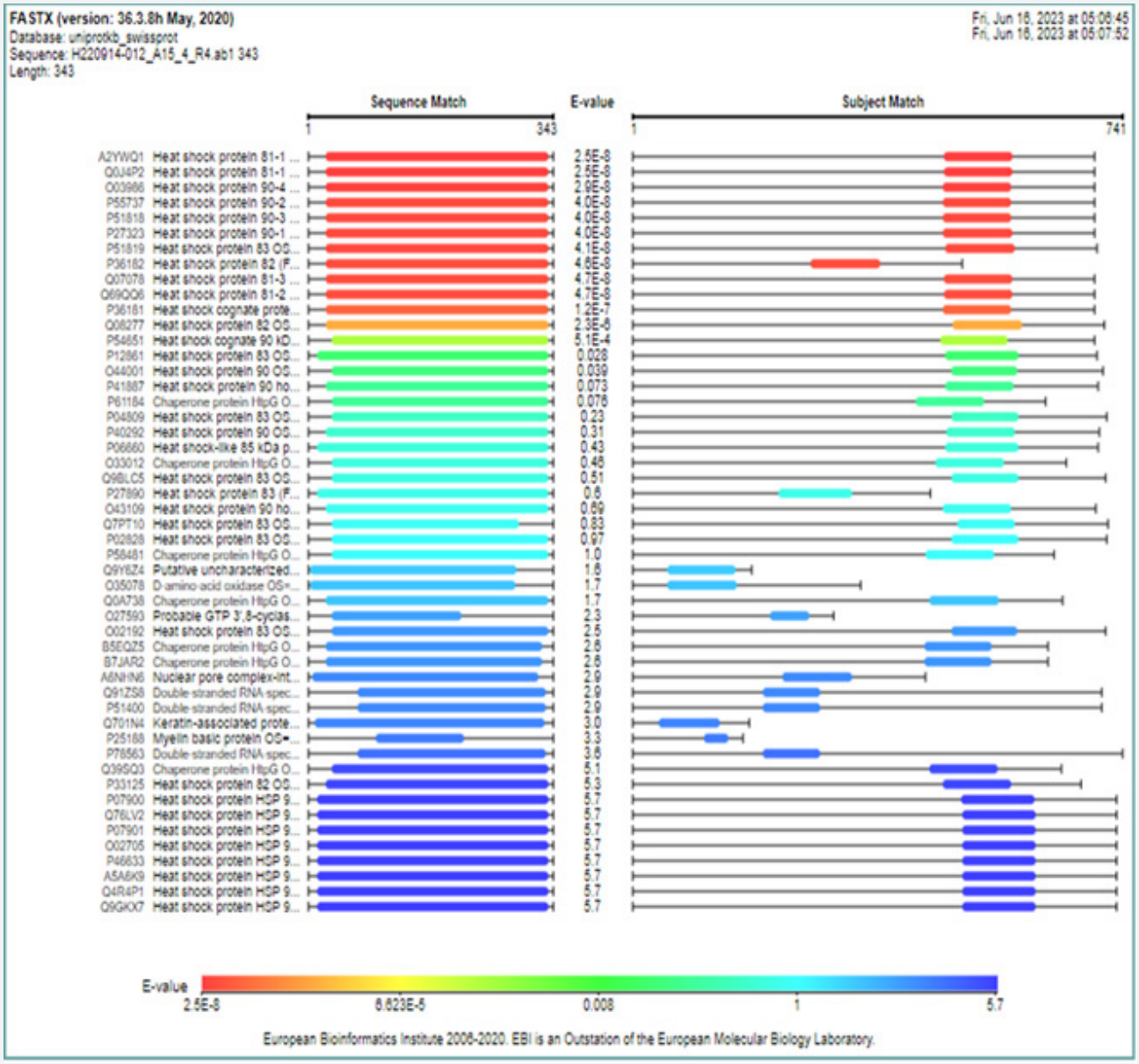

Figure 8: visual output of FASTA alignment via protein similarity search of FASTX for DNA Sequence through European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI).

D. Hsp90 gene conserved domain

It also contains the MDLERVVMSDRGADSPCCLTLGMTSH domain which represents a conserved domain of the Hsp90 gene superfamily (marker-signature). Conserved domains represent a main or fundamental feature for sequence recognition. In general, statistically significant similarity scores can be used to infer homology with high levels of confidence; the major exception is low complexity repeated domains in proteins. The converse is not true; the absence of significant similarity does not guarantee nonhomology. Homologous sequences share a common ancestor, and thus a common protein fold. Depending on the evolutionary distance and divergence path, two or more homologous sequences may have very few conserved residues (Figure 10). Many diverse protein families contain members with low pairwise sequence similarity, but they can be identified because homology is transitive: if A is related to B and B is related to C, A must be related to (homologous to) C, even if they do not share significant sequence similarity [32]. For single-domain proteins, the end of the alignment may coincide with the ends of the proteins, but for domains

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

that are found in different sequence contexts in different proteins, the alignment should be limited to the homologous domain, since the domain homology is provides the sequence similarity captured in the score. When local alignments end within a protein, the ends of the alignment can depend on the scoring matrix used to calculate the score. Scoring matrices like BLOSUM62, which is used by BLASTP, or BLOSUM50, which is used by SSEARCH and FASTA, are designed to detect very distant similarities, and have relatively low penalties for mismatched residues [29]. Also, EMBL - EBI predicted the whole sequence through FAST X AS Cytosolic HSP90 According to family and domain prediction based on blast x alignment shown in Figure 7, it predicted heat shock protein 90family through HAMAP, PANTHER, PFAM and PIRSF protein features and C-terminal conserved domain of HSP90 which marked by MKAQALR and MEEVD through CATCHGENE. Without further ado, primers used for sequencing succeeded in isolating a very conserved region, sequencing of the reverse direction provides alignment able information representing an access to unknown ambiguous genomes. We should investigate more the ability to purify low quality ambiguous sequencing data through detecting conserved regions alignment or comparisons to databases to find more reliable sequence segments; further, increase the probabilities or potentials to construct a more specific oligonucleotide primer. Also conserved sequences can be used in an effective way to get into limited information sequences or genomes, giving precious information. This yielded information can be used to design more specific primers which give a better chance for regular analysis such as sequencing, primer walking, regular PCR…. Etc. (Figure 11).

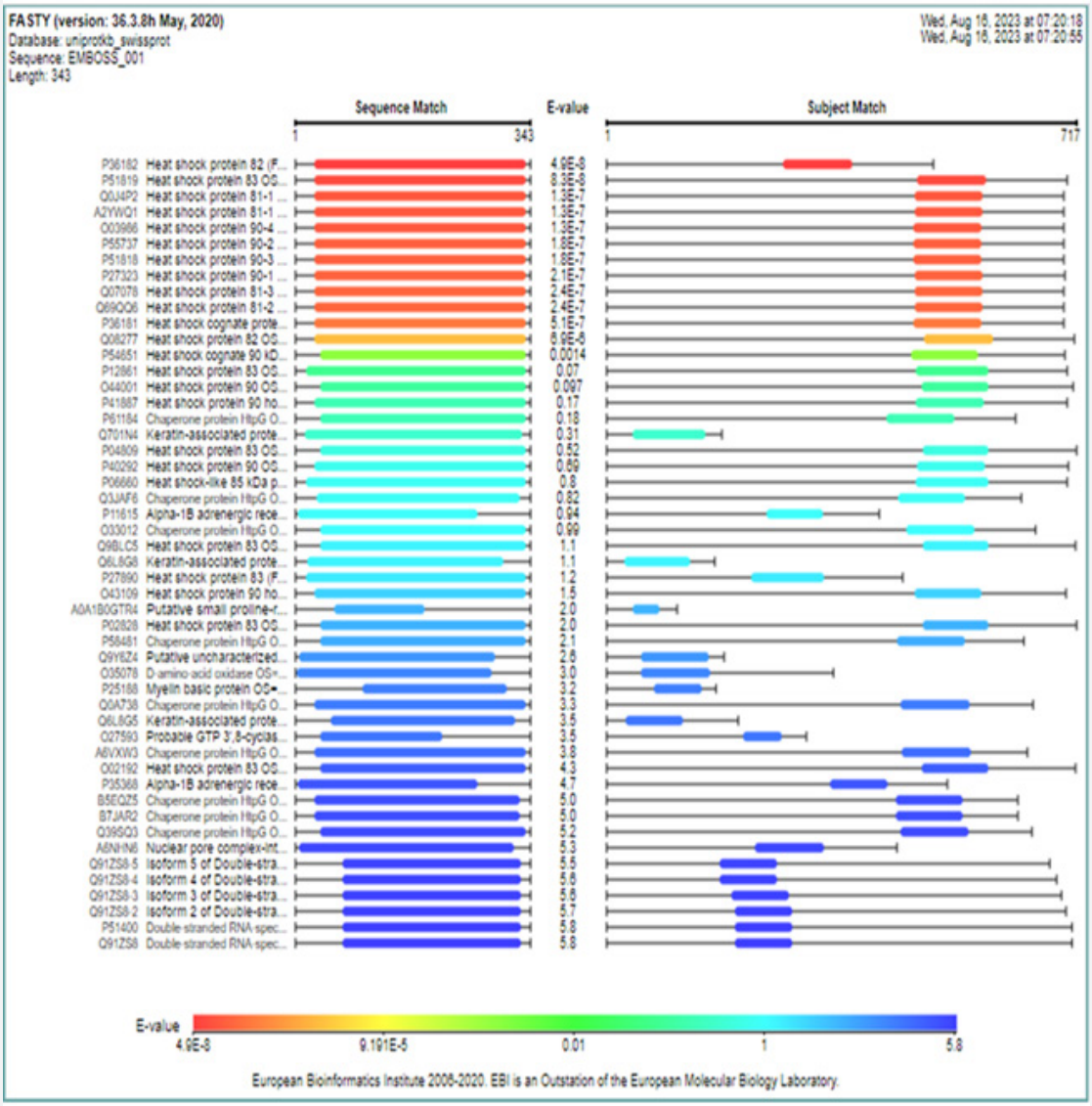

Figure 9: visual output of FASTA alignment via protein similarity search of FASTY for DNA Sequence through European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI).

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

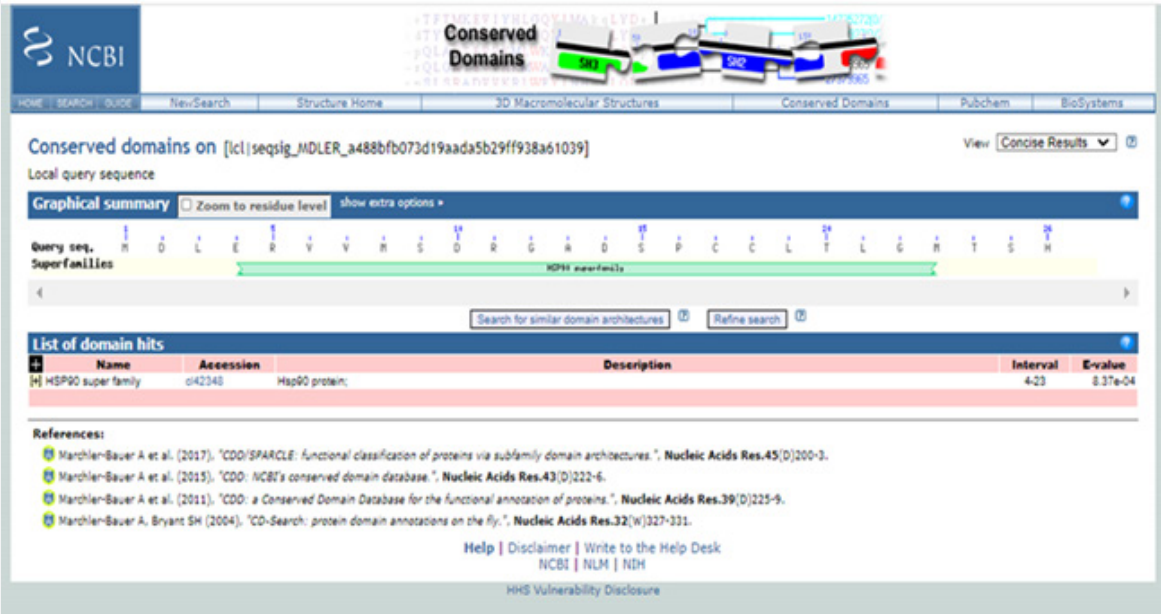

Figure 10: explained the detected conserved domain (ERVVMSDRGADSPCCLTLGM) represent hsp90 signature via NCBI.

E. Screening *Salvadora presica* response to different salinity levels

a. Real-time quantitative RT-PCR (qRT-PCR) analysis

Quantitative real-time reverse transcriptase PCR (qRT-PCR) is a sensitive and powerful technique for measuring differential gene expression and changes in gene expression induced by abiotic stresses.

The 90-kD heat shock protein (HSP90s) are a conserved class of chaperones that play critical roles during the normal life cycle of plants. HSP90s proteins are particularly involved in the regulation of biotic and abiotic stress responses. In this research the potential roles of the *Salvadora presica* Hsp90 gene were investigated to evaluate *Hsp90* expressions under normal conditions and different salinity levels.

The assessment of the expression of the Hsp90 gene was performed using real time-polymerase chain reaction (RT-PCR). Stress treatments were done on seedlings irrigated using 100 Mm NaCl,250 mM NaCl, 500 mM NaCl and 750 mM NaCl for each treatment within 10 days.

Salvadora presica gene expression profile showed that HSP90 gene reached the highest level at 500 mM then it started to decline (Chatr 1).

Surprisingly 100mM expression profile shown a downregulation of *salvadora presica* Hsp90.1 gene. 1 may be because of its nature as a halophyte so it indicates that this concentration may be more favorable to plants even more than the control. Obligate halophytes cannot survive in freshwater permanently, salt is necessary for their growth and a physiological requirement, and control conditions can represent stressful agents for them [33b]. We need to investigate more how *salvadora presica* which is known as a facultative halophyte respond to a control state compared with low salt concentrations, to find the best growth conditions, even though control or low salt concentrations do not represent serious stressful conditions.

Also, some previous studies indicated that low level of NaCl stimulates plant growth even in glycophyte [34]. Each according to their scale or spectrum.

Generally, salt stress affects plants in two phases. In the first phase, it causes osmotic stress that reduces water extractability, leading to growth retardation. In the second phase, harmful levels of ions accumulate in plant cells, resulting in toxicity through enzyme inactivation, inhibition of protein synthesis, and disruption of the plasma membrane [35]. As represented by the expression graph the first phase (*Hsp90* gene upregulation) went up to 500mM, and then the second phase (Hsp90 gene downregulation) effect started to appear at 750mM. In terms of salinity stress tolerance, plants can be divided into halophytes and glycophytes. Most crop species are glycophytes. Many glycophytes are particularly intolerant to salt, being inhibited by NaCl concentrations around 25-50 mmol L-1 [36]. which indicates that *salvadora presica* can tolerate at least 10x more than a 50mM tolerant glycophyte.

b. Proline

Proline assessment expressed two phases first increase of prolines up to 500mM Salinity then decline at 750 mM (Chart 2).

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

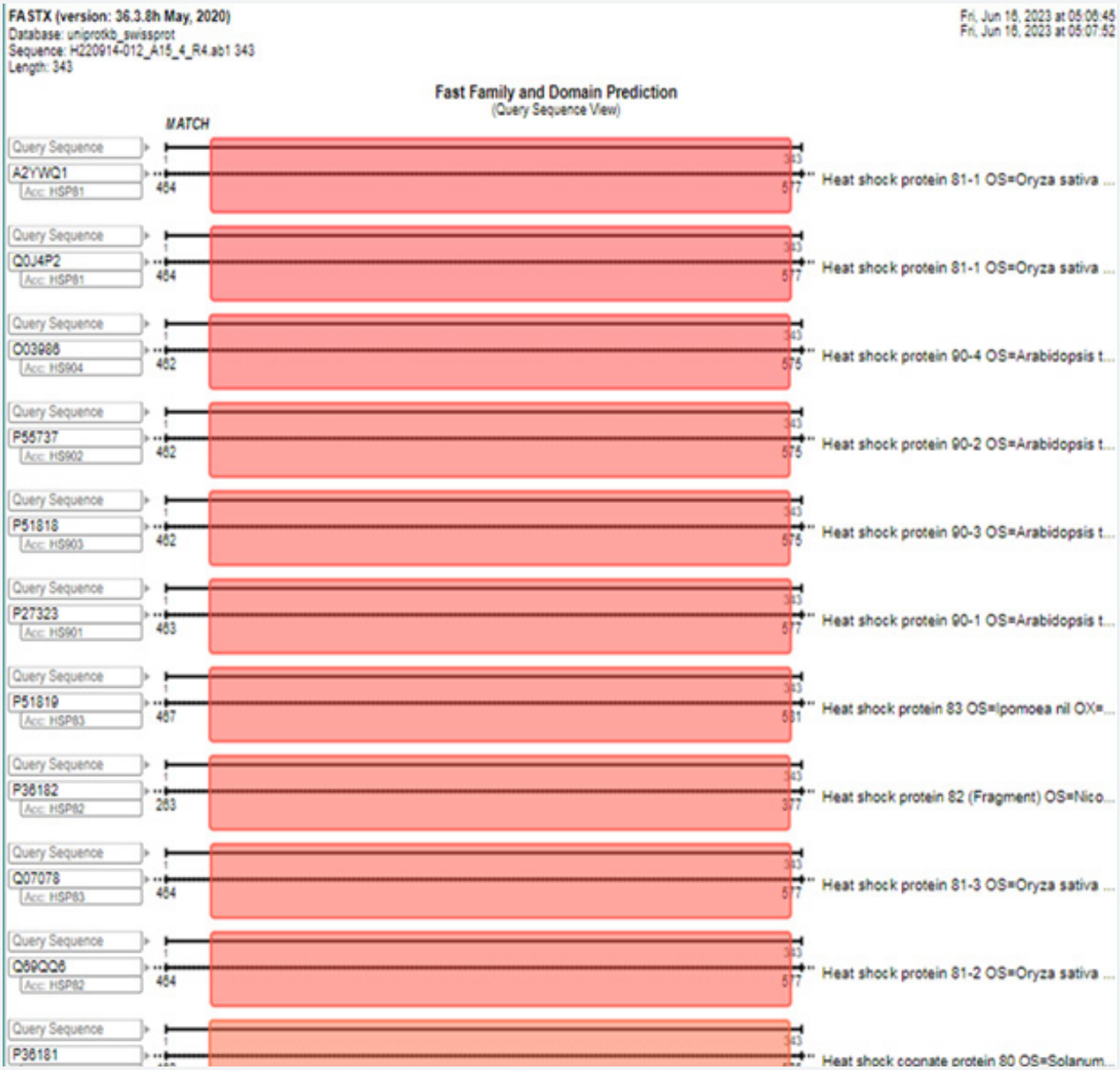

Figure 11: Functional predictions of FASTA alignment via protein similarity search of FASTX for DNA Sequence Through European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI).

In many plants, free proline accumulates in response to a wide range of stresses, including biotic and abiotic, such as salinity stress, water deficit stress, extreme temperatures, heavy metal toxicity, pathogen infection, nutrient deficiency, and UV radiation. Proline accumulation has been suggested to result from (a) decreased proline degradation; (b) increased proline biosynthesis; (c) lower proline utilization, and (d) hydrolysis of proteins [37]. Proline is a common phenomenon in plants. Besides its role as an osmolyte, proline contributes to scavenging ROS, stabilizing subcellular structures, modulating cell redox homeostasis, supplying energy and functioning as a signal. Although proline accumulation is a common response to salt stress, the extent of its accumulation varies between tolerant and sensitive genotypes, proline accumulation increases greatly within the tolerant genotype, accumulated preferentially in leaves to maintain chlorophyll level and cell turgor to protect photosynthetic activity under salt stress. Proline has also a potential role in scavenging ROS products. The accumulation of proline in plants under stress is caused either by the

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

induction of expression of proline. biosynthesis genes or by the repression of the genes of its degradation pathway (PDH silencing) as indicated by [38]. This explains raising concentrations of proline up to 500mM salinity [39]. explained. that exogenous proline enhances plant growth, it needs to be investigated whether indigenous prolines halophytes produced under low salt concentration, can have the potential to enhance plant growth, the same as exogenous prolines. The Concentration of 750 mM shows a noticeable decrease in proline may be due to degradation, aggregation, and misfolding due to chaperoning mechanisms becoming weaker or due to degradation and getting rid of defective prolines through co-chaperoning. in general, it become more crucial to save cell energy directed towards fix defected prolines.

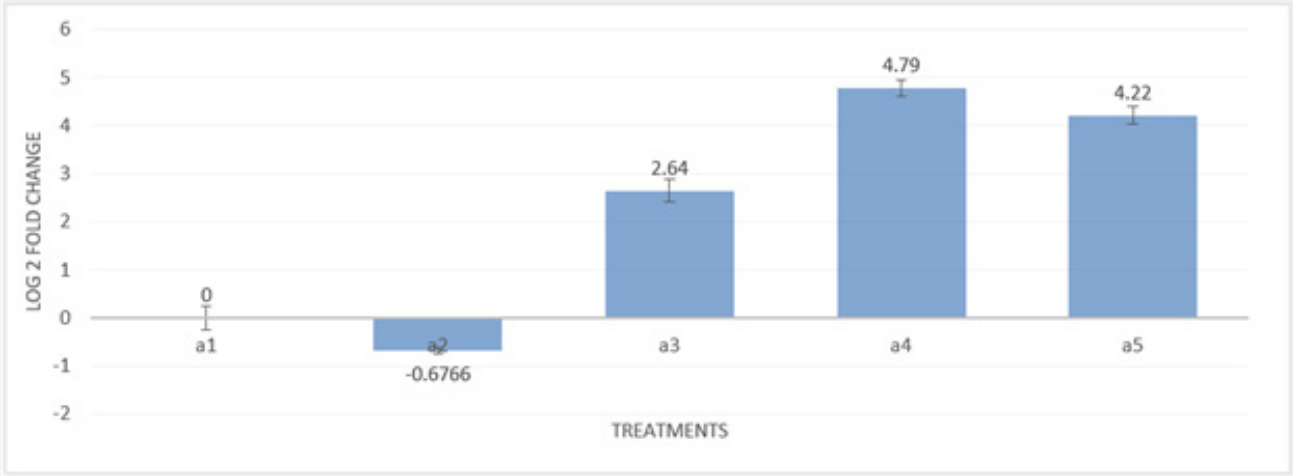

Chart 1: Salvadora presica Hsp90.1 gene expression under different salinity concentrations (a1=control; a2=100mM; a3=250mM; a4=500mM and a5=750mM).

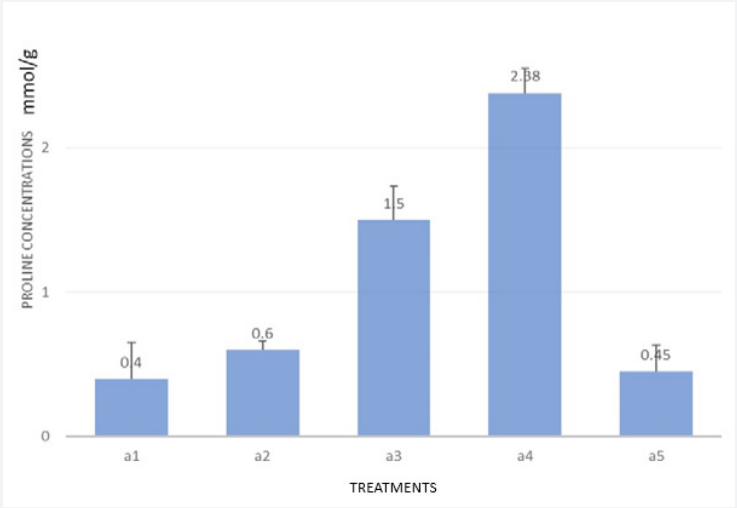

Chart 2: Proline profile of Salvadora presica under different salinity condition a1=control; a2=100mM; a3=250mM; a4=500mM and a5=750mM).

In conclusion proline profile expressed two phases, steady increase till 500mM concentration then decline at 750mM of NaCl. From 250 mM to 500 mM show steady upregulation of Hsp90.1 and an increase of proline content at 750 level molecular (Hsp90.1) start to deterioration and a quite decrease of proline content which represents the second phase. Proline and Hsp90.1 expression profiles indicated that NaCl caused a significant increase in proline content (stress indicator parameter) and upregulation in heat shock protein gene expression levels (adaptive mechanisms). Results suggest that *Salvadora prescia* NaCl stress tolerant /adapt up to 500mM.

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

Conclusion

This study succeeded in detecting *Salvadora presica* partial DNA sequence of HSP90 gene within its sequence of the conserved domain which represents the Hsp90 super family signature. Also screening *salvadora presica* responses to high salinity based on the molecular basis (expression of *HSP90.1*) and proline levels indicated that it could adapt salinity up to 500mM of Nacl.

References

1. Al Khateeb W, Muhaidat R, Alahmed S, Al Zoubi MS, Al-Batayneh KM, et al. (2020) Heat shock proteins gene expression and physiological responses in durum wheat (Triticum durum) under salt stress. Physiol Mol Biol Plants 26(8): 1599-1608.
2. Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207.
3. Buchner J (1999) Hsp90 & Co.- a holding for folding. Trends Biochem Sci 24(4): 136-141.
4. Chen S, Polle A (2010) Salinity tolerance of Populus. Plant Biol (Stuttg)12(2): 317-333.
5. Crisp Michael D, Weston Peter H (2010) Telopea speciosissima (Sm) R Br New South Wales Flora Online.
6. Debnath M, Bisen PS (2008) Jatropha curcas L a multipurpose stress resistant plant with a potential for ethnomedicine and renewable energy. Curr Pharm Biotechnol 9(4): 288-306.
7. Erjavec MS (2020) Annealing Temperature of 55˚C and Specificity of Primer Binding in PCR Reactions.
8. Fahmideh L, Fooladvand Z (2018) Isolation and Semi Quantitative PCR of Na^+/H^+ Antiporter (SOS1 and NHX) Genes under Salinity Stress in Kochia scoparia. Biol Proced Online 20: 11.
9. Flowers TJ, Muscolo A (2015) Introduction to the Special Issue: Halophytes in a changing world. AoB Plants 7: plv020.
10. França L, Carrilho E, Kist T (2002) A review of DNA sequencing techniques. Q Rev Biophys 35(2): 169-200.
11. Gharsallah C, Fakhfakh H, Grubb D, Gorsane F (2016) Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. AoB Plants 8: plw055.
12. Gish W, States D J (1993) Identification of protein coding regions by database similarity search. Nat Genet 3(3): 266-272.
13. Gonzalez MW, Pearson WR (2010) Homologous over-extension: a challenge for iterative similarity searches. Nucleic Acids Res 38(7): 2177-2289.
14. Hongqiao L, Suyama A, Mitani-Ueno N, Hell R, Maruyama-Nakashita A (2021) A Low Level of NaCl Stimulates Plant Growth by Improving Carbon and Sulfur Assimilation in Arabidopsis thaliana. Plants (Basel) 10(10): 2138.
15. Khatak M, Khatak S,Siddqui A A, Vasudeva N, Aggarwal A,et al. (2010) Salvadora presica. Pharmacogn Rev4(8): 209-214.
16. Lobo I (2008) Basic Local Alignment Search Tool (BLAST). Nature Education 1(1): 215.
17. Lopez D L, Gimeno V, Lidon V, Simón I, Martínez V, et al. (2012) The tolerance of Jatropha curcas seedlings to NaCl: an ecophysiological analysis. Plant Physiol Biochem 54: 34-42.
18. Lausser L, Kestler HA (2014) Fold Change Classifiers for the Analysis of Gene Expression Profiles. In: Gaul W, Geyer SA, Baba Y, Okada A (Eds.), German Japanese Interchange of Data Analysis Results. Studies in Classification, Data Analysis, and Knowledge Organization. Springer, Cham.
19. Malhotra K, Foltz L, Mahoney W C, Paula A Schueler (1998) Interaction and effect of annealing temperature on primers used in differential display RT-PCR. Nucleic Acids Res 26(3): 854-856.
20. Momenpour A, Imani A (2018) Evaluation of salinity tolerance in fourteen selected pistachio (Pistacia vera L.) cultivars. Advances in Horticultural Science 32(2): 249-264.
21. Monfared M, Sampsampour D, Sirchi SRG, Sadeghi F (2018) Assessment of genetic diversity in salvadora presica L. based on intersimple sequence repeat (ISSR) genetic marker. J Genet Eng Biotechnol 16(2): 661-667.
22. Parida AK, Veerabathini SK, Kumari A, Agarwal PK (2016) Physiological, Anatomical and Metabolic Implications of Salt Tolerance in the Halophyte Salvadora persica under Hydroponic Culture Condition. Front Plant Sci 7: 351.
23. Pearson W R (1996) [15] Effective protein sequence comparison. Methods Enzymol 266: 227-58.
24. Pearson RW, Wood T, Zhang Z, Miller W (1997) comparison of DNA sequences with protein sequences 46(1): 24-36.
25. Pearson R W (2014) An Introduction to Sequence Similarity (Homology) Searching. Curr Protoc Bioinformatics 3: 311-318.
26. Pruitt K, Brown G, Tatusova T, et al. (2002) The Reference Sequence (RefSeq) Database. In: McEntyre J, Ostell J (Eds.), The NCBI Handbook. Bethesda (MD): National Center for Biotechnology Information (US), Chapter 18.
27. Rady MM, Taha S R, Mahdi AHA (2016) Proline enhances growth, productivity, and anatomy of two varities of lupines termis l. Grown under salt stress. South Africa Journal of Botany 102: 221-227.
28. Rangani J, Parida A K, Panda A, Kumari A (2016) Coordinated changes in antioxidative enzymes protect the photosynthetic machinery from salinity induced oxidative damage and confer salt tolerance in an extreme halophyte Salvadora persica L. Front Plant Sci 7: 50.
29. RK R, N S N, S P A, Sinha D, Veedin Rajan VB, et al. (2012) HSPIR: a manually annotated heat shock protein information resource. Bioinformatics 28(21): 2853-2855.
30. Solares CA, Hall JR, Xue X, Rise ML (2022) Reverse Transcription-Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) for Gene Expression Analyses. Methods Mol Biol 2508: 319-340.
31. Song S Q, Lei Y B, Tian X R (2005) Proline Metabolism and Cross-Tolerance to Salinity and Heat Stress in Germinating Wheat Seeds. Russian journal of plant physiology 52(6): 793-800.
32. Schmittgen T, Livak K (2008) Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3(6): 1101-1108.
33. Shendure Jay, Balasubramanian Shankar, Church George M, Gilbert Walter, Rogers Jane, et al. (2017) DNA sequencing at 40: past, present, future. Nature 550(7676): 345-353.
34. Stechmann A, Cavalier-Smith T (2003) Phylogenetic Analysis of Eukaryotes Using Heat-Shock Protein Hsp90. J Mol Evol 57(4): 408-419.
35. SS Tahir, MT Rajput, F Korejo (2010) A new species of Salvadora (Salvadoraceae) from Sindh, Pakistan. Pak J Bot 42: 63-66.
36. Udvardi K M, Czechowski T, Scheible WR, Eleven Golden Rules of Quantitative RT-PCR. Plant Cell 20(7): 1736-1737.
37. Wang GF, Wei X, Fan R, Zhou H, Wang X, et al. (2011) Molecular analysis of common wheat genes encoding three types of cytosolic heat shock protein 90 (Hsp90): functional involvement of cytosolic Hsp90s in the

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363

control of wheat seedling growth and disease resistance. New Phytol 191(2): 418-431.

38. Wang W, Yan Z, You S, Zhang Y, Chen L, et al. (2011) Mangroves: obligate or facultative halophytes A review. Trees 25: 953-963.

39. Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9(5): 244-252.

40. White BA (1993) PCR Protocols. Current Methods and Applications. 1st ed. Totowa: Humana Press Inc.

41. Honghong Wu (2018) Plant salt tolerance and Na^+ sensing and transport, The Crop Journal 6(3): 215-225

42. Zainordin F Z, San T C, Ahmad A (2021) Isolation, Molecular characterization, and expression level of cytosolic Heat-Shock protein 90 (HSP90) genes of Cryptocoryne Ciliata, a halophyte plant. Journal of Sustainability Science and Management 16(7): 31-45.

DOI: 10.19080/ARTOAJ.2023.28.556363

How to cite this article: Noha M E M H, Moustafa M E, Mounir M, Hemaid I A S, Mohamed M Y. Molecular Characterization and Expression Profiling of Cytosolic Hsp90 Gene in the Facultative Halophyte *Salvadora presica* under Different Salinity Levels. Agri Res& Tech: Open Access J. 2019; 28(1): 556363. DOI:10.19080/ARTOAJ.2023.28.556363