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Research Article
Differential Expression of Expressed Sequence Tags (ESTs) Regulated in Response to Salt Stress Conditions in Date Palm

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Abstract

Background and Objective: *Phoenix dactylifera* L. (Date palm) is the most important crop in several drought regions of North Africa, the Middle East and Arabian Peninsula. Salinity is one of the main problems that can severely affect the production of Date palm. So, the objective of this study was to investigate biochemical and physiological basis for date palm salinity tolerance, isolate and characterize Expressed Sequence Tags (ESTs) involved in response to salinity stress conditions by using DD-PCR technique. **Materials and Methods**: In current research, some biochemical and physiological analysis was done (chlorophyll pigments , malondialdehyde (MDA) concentration and the ratio of K^+/Na^+ and also DD-PCR was used as molecular aspects to detect ESTs related to salt tolerance in two Egyptian Date palm cultivars (cv. Bertamoda and cv. Malkabi) at seedling stage. **Results:** The results of chlorophyll pigments showed reduction in salt stressed plants, while, malondialdehyde (MDA) concentration and the ratio of K^+/Na^+ were increased. The DD-PCR results revealed the appearance of 17 (7 ESTs from Bertamoda and 10 ESTs from Malkabi) up-regulated genes in salt-stressed plants compared to control. **Conclusion:** Bertamoda cultivar is more tolerant to salt stress than Malkabi cultivar, because it has the ability to protect the photosynthetic pigments, absorb low amounts of Na^+, minimize the production of MDA and upregulated 17 genes that controlling the expression of many enzymes and hormones responsible for many metabolic pathways that could be the way for tolerating and minimizing the bad effects of salt stress.

Key words: Date palm, salt stress, Expressed Sequence Tags (ESTs), chlorophyllous pigments, malondialdehyde

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Phoenix dactylifera L. belongs to family Arecaceae, that included about 200 genera and 2,500 species[1]. It is the most important crop in several drought regions of North Africa, the Middle East and Arabian Peninsula. About 75% of world annual date production is produced from Egypt, Iran, Saudi Arabia, Algeria, Iraq and Pakistan[2]. The complexity of date palm cultivation requires continued research to unravel different aspects related to palm tree health and growth. A very important factor to be considered is the abiotic stress especially water scarcity and salinity[3].

Salinity is one of the main problems that can severely affect the production of crops[4] and cause desertification of major agricultural areas[5]. It is expected that there will be about 35% land loss in the next 25 years because of salinity[6].

Salinity has bad effect on the nutrient uptake in which potassium uptake reduced by sodium and nitrate uptake reduced by chloride[7,8]. Salinity negatively affects the photosynthetic procedures, including stomatal closure and chlorophyll content[9].

Malondialdehyde (MDA) is used as an indicator for lipid peroxydation and oxidative stress damage[10], while salinity cause over production of MDA content[11]. Stressed plants produce Reactive Oxygen Species (ROS) that include superoxide (O_2^-), hydrogen peroxide (H_2O_2) and osmosis stress[12] these molecules may attack DNA responsible for producing proteins and unsaturated lipids membranes[13,14].

Different date palm varieties show different levels of tolerance to salinity[9]. Previous studies on salt adaptation focused on the genetic differentiation by using DNA markers, such as RAPD (Random Amplified Polymorphic DNA)[15,16], AFLP (Amplified Length Polymorph)[17,18] and microsatellites[19]. However, previous studies didn't successfully identified/characterized any functional genes related to salt-tolerance trait in date palm. Date palm represents a treasure trove of novel genetic resource for salinity tolerance, yet mechanisms concerning salt tolerance in date palm are still unknown[3].

Differential Display Polymerase Chain Reaction (DD-PCR) is a method designed to identify differentially induced or expressed genes and successfully identified new genes in various plants[20]. It has been widely used for the isolation of a large number of differentially Expressed Sequenced Tags (ESTs) due to the simplicity and accuracy of comparing the transcripts in several treatments simultaneously[21-23]. It used for screening both down-regulated and up-regulated transcripts in multiple cell populations under different environmental and developmental conditions[24].

Therefore, the objective of this study was to investigate the biochemical and physiological basis for date palm salinity tolerance, isolate and characterize Expressed Sequence Tags (ESTs) involved in response to salinity stress conditions by using DD-PCR technique.

MATERIALS AND METHODS

Plant material and salinity experiment: *Phoenix dactylifera* L. (Date palm) cultivars Bertamoda and Malkabi were used as the plant material for biochemical, physiological and molecular analysis. These cultivars were obtained from Date Palm Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Bertamoda and Malkabi were cultivated under greenhouse controlled conditions with irrigation twice/week using normal tap water supplemented with Nitrogen, Phosphorus and Potassium (NPK) for four weeks. Each cultivar has divided into 3 groups/treatments, with three replicates for each treatment. The treatments included Control (irrigated with tap water), NaCl treatment (solution adjusted to 12000 ppm) and Diluted Sea Water (DSW) treatment (solution adjusted to 12000 ppm). The seedlings were irrigated for a week using 250 mL/time every 48 h. Then, the samples of leaves tissues were harvested, frozen in liquid nitrogen and stored at -80°C. Two types of analysis were performed; biochemical and physiological analysis (MDA production, Chlorophyllous pigments concentration and Na^+/K^+ ratio) and molecular analysis by using DD-PCR. The study was carried out at Genomics and Proteomics Laboratory in Department of Nucleic Acid and Protein Structure, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt from June 2018-January 2019).

Biochemical and physiological analysis

Estimation of MDA production: The level of lipid peroxides was determined in leaves as malondialdehyde (MDA) content by the thiobarbituric acid (TBA) reaction according to Heath and Packer[25].

Concentrations of chlorophyllous pigments: Chlorophyll was extracted by incubating 1 g of leaves taken from control and treated plants in N,N -dimethylformamide (DMF) according to Moran[26]. Using spectrophotometer, the absorbance of each sample was measured at three wave lengths: 625, 647 and 664 nm. The obtained readings were calculated to determine the chlorophyll a, chlorophyll b and total chlorophyll concentrations.

Analysis of K^+/Na^+: Half gram of the leaves was digested in sulphuric-perchloric acids mixture ($HClO_4$ and H_2SO_4) acids according to Chapman and Pratt[27]. Then total K^+ and Na^+ were determined by Flame photometer as described by Jackson[28].

Molecular analysis

RNA extraction and cDNA synthesis: Total RNAs was extracted from control and treated seedlings according to the procedure of Chomczynski[29] using TriPure isolation reagent (Roche Molecular Biochemicals, Germany). The RNA was quantified with NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific Inc.). All samples were adjusted to a concentration of 1 μg μL^{-1} for subsequent analyses. First and second cDNA strands were synthesized using ImProm-II™ Reverse Transcription System (Promega, Madison, Wisconsin, USA).

Differential display-polymerase chain reaction (DD-PCR) analysis: Differential display technique was employed according to Liang and Pardee[30] with some modifications. To isolate novel ESTs responsible for salinity tolerance in *Phoenix dactylifera* seedlings.

Anchor primer T_{11} A in combination with some arbitrary primers were used. The sequence names of Anchored and Arbitrary primers and their sequences for DD-PCR analysis are as follow: Anchored primer; T11A (5'-TTT TTT TTT TTA-3'), Arbitrary primers; AP1 (5'-AAG CTT GAT TGC C-3'), AP2 (5'-AAG CTT CGA CTG T-3'), AP3 (5'-AAG CTT TGG TCA G-3'), AP4 (5'-AAG CTT CTC AAC G-3'), AP5 (5'-AAG CTT AGT AGG C-3'), AP6 (5'-AAG CTT GCA CCA T-3'), AP7 (5'-AAG CTT AAC GAG G-3'), AP8 (5'-AAG CTT TTA CCG C-3'), AP9 (5'-AAG CTT ACG CAA C-3)'. All anchored and arbitrary primers were synthesized in (Euro-fins MWG Operon, Huntsville, Germany). Go *Taq*® Flexi DNA polymerase (Promega, Madison, Wisconsin, USA) was used for amplification. Separation of amplified fragments was carried out on 6% polyacrylamide gels using Sequi-Gen® Sequencing Cell (Bio-Rad Laboratories, Hercules, California, USA). The gels were silver stained using the silver sequence kit (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions.

Isolation and re-amplification of differential fragments: Sterile scalpel blades were used to cut the desired bands from the gel. Gel slices were incubated in 50 μL DD H_2O at 65°C for 30 min and then left at room temperature for elution. Three microliter of the aliquot were used for re-amplification in a total volume of 25 μL, using the same set of corresponding primers. The reactions of PCR and the re-PCR for the selected DD fragments were carried out in a GeneAmp® PCR System 9700 instrument, programmed for 94°C for 1 min (1 cycle); 94°C for 30 sec, 38°C for 2 min, 72°C for 30 sec (40 cycles); 72°C for 5 min (1 cycle), then held at 4°C. The PCR products for re-amplification were checked in a 2% agarose gel.

Nine combinations for both cultivars were used but, the reproducible combinations for Bertamoda cultivar were three combinations (PdT_{11}A/AP1, PdT_{11}A/AP6 and PdT_{11}A/AP7) and two combinations for Malkabi cultivar (PdT_{11}A/AP1and PdT_{11}A/AP7).

Sequencing of differential fragments and data analysis: The re-amplified DD fragments were sequenced using ABI PRISM BigDye® terminator cycle sequencing ready reaction kit (Applied Biosystems, USA), in conjunction with ABI PRISM 3730xl DNA Analyzer (Applied Biosystems, USA). About 17 differential fragments were subjected for DNA sequencing analysis. The sequences were analyzed using BLAST algorithm[31], BLASTX, BLASTN and dbEST against NCBI database to compare the differential fragments with similar fragments against date palm genome (http://www.ncbi.nlm.nih.gov). When no homology was found in the non redundant protein or DNA databases, in an Expressed Sequence Tag (EST) database of the *Phoenix dactylifera* for significant homologues according to E-value was looked.

RESULTS

Biochemical and physiological analysis

MDA production by measuring lipid peroxidation: In this study, the findings revealed highly significant increase of MDA content in the NaCl and DSW treated samples of both cultivars when compared to the control, especially in Malkabi cultivar (Table 1).

Table 1: MDA concentration in two cultivars of date palm under salt stress conditions

Phoenix dactylifera cultivars	Treatments	Concentration of MDA μmol/g fw
Bertamoda	Control	0.760
	NaCl	1.470^{+HS}
	DSW	2.200^{+HS}
L.S.D. (5%)		0.137
L.S.D. (1%)		0.196
Malkabi	Control	3.500
	NaCl	4.700^{+HS}
	DSW	5.560^{+HS}
LS.D. (5%)		0.374
L.S.D. (1%)		0.538

+HS: Highly significant change

Table 2: Chlorophyllous pigments content (μg mL^{-1}) in two cultivars of date palm under salt stress conditions

Phoenix dactylifera cultivars	Treatment	Chlorophyll a (Chl a)	Chlorophyll b (Chl b)	Total chlorophyll
Bertamoda	Control	12.546	3.812	0.547
	NaCl	12.363^{NS}	3.870^{NS}	0.558^{NS}
	DSW	12.238^{NS}	3.723^{NS}	0.469^{-HS}
L.S.D. (5%)		0.970	0.298	0.041
L.S.D. (1%)		1.394	0.428	0.060
Malkabi	Control	14.077	4.526	0.879
	NaCl	12.745^{-S}	3.973^{-HS}	0.323^{-HS}
	DSW	10.639^{-HS}	3.193^{-HS}	0.388^{-HS}
L.S.D. (5%)		0.993	0.313	0.052
L.S.D. (1%)		1.428	0.449	0.074

-HS: Highly significant change, S: Significant change, NS: Non-significant change

Table 3: Concentrations of K^+ and Na^+ in two cultivars of date palm under salt stress conditions

Phoenix dactylifera cultivars	Treatment	K^+	Na^+	K^+/Na^+
Bertamoda	Control	0.39	0.69	0.57
	NaCl	0.40^{NS}	0.73^{NS}	0.55^{NS}
	DSW	0.32^{-HS}	0.69^{NS}	0.46^{-HS}
L.S.D. (5%)		0.029	0.055	0.042
L.S.D. (1%)		0.042	0.079	0.060
Malkabi	Control	0.33	0.43	0.77
	NaCl	0.09^{-HS}	0.13^{-HS}	0.69^{-HS}
	DSW	0.19^{-HS}	0.38^{-HS}	0.50^{-HS}
L.S.D. (5%)		0.020	0.029	0.053
L.S.D. (1%)		0.029	0.042	0.076

-HS: Highly significant change, S: Significant change, NS: Non-significant change

Determination of chlorophyllous pigments: Results showed a significant decrease in chlorophyll a, b and protochlorophyll (Pchl) in salt-treated samples compared to control in both cultivars Bertamoda and Malkabi. In NaCl and DSW treatments, the results revealed highly significant reduction in the chlorophyll content in Malkabi than in Bertamoda (Table 2).

K^+ and Na^+ analysis: The cultivars under study showed variable response for each treatment. In Bertamoda, DSW samples showed the same Na^+ content as the control sample. Also, the NaCl treated sample showed non significant increase in K^+ content when compared with control. On the other hand, in Malkabi both treatments showed highly significant reduction in Na^+ content especially the NaCl treated samples which showed a huge drop in both Na^+ and K^+ values. In Bertamoda the reduction was just in K^+ while the Na^+ is not affected, while Malkabi showed highly significant reductions in both K^+ and Na^+ and the ratio of K^+/ Na^+ (Table 3).

Molecular analysis

Expression pattern of DD transcripts: A number of 17 fragments were successfully identified as a result of salinity conditions, 7 of which were from Bertamoda cultivar and 10 were from Malkabi cultivar. These fragments varied in length from 212-1361 bp.

Sequence analysis of the DD transcripts for Bertamoda and Malkabi cultivars: The up-regulated ESTs in response to salt stress conditions were identified for both cultivars Bertamoda and Malkabi (Table 4). To facilitate the subsequent analysis with the DD fragments, a specific nomenclature was adopted based on the primers combinations used in the amplification and the band number on the gel. For example, Bertamoda cultivar cDNA: $PdT_{11}A/AP1$-1B refers to *Phoenix dactylifera* amplified using anchor primer $T_{11}A$ in combination with arbitrary primer AP1, B for Bertamoda cultivar (Fragments from 1B to 7B); Malkabi cultivar cDNA: $PdT_{11}A/AP1$-1M refers to *Phoenix dactylifera* amplified using anchor primer $T_{11}A$ in combination with arbitrary primer AP1, M for Malkabi cultivar (Fragments from 1-10 M).

No significant similarity: According to Table 4, it can be distinguish two ESTs ($PdT_{11}A/AP1$- 2B and $PdT_{11}A/AP7$- 4M) with no significant sequence similarity to known sequences in the genbank.

DISCUSSION

The result of lipid peroxidation under salinity stress (Table 1) indicated that cell was damaged through the degradation of cellular Polyunsaturated Fatty Acids (PUFA)

Table 4: BLAST analysis of the sequenced DD transcripts as compared to date palm genome and KEGG pathways

Fragment No.	No. of bases	Homology accession No.	Homology	KEGG No.	KEGG pathways	Identified (%)
$PdT_{11}A/AP1-1B$	212	XM_008779600.2	Predicted: *Phoenix dactylifera* inositol monophosphatase 3 (LOC103697686), transcript variant X4, mRNA	103697686	(pda00053) Ascorbate and aldarate metabolism (pda00562) Inositol phosphate metabolism (pda01100) Metabolic pathways (pda01110) Biosynthesis of secondary metabolites (pda04070) Phosphatidylinositol signaling system	100
$PdT_{11}A/AP1-2B$	294		No significant similarity found			
$PdT_{11}A/AP6-3B$	582	XP_008793954.1	Predicted: Pyrophosphate-energized vacuolar membrane proton pump-like [*Phoenix dactylifera*]	103710117	(pda00190) Oxidative phosphorylation	63
$PdT_{11}A/AP6-4B$	702	XP_008803426.1	Predicted: Phospholipid-transporting ATPase 3 [*Phoenix dactylifera*]	---	Unknown	57
$PdT_{11}A/AP7-5B$	517	XM_008808043.2	Predicted: *Phoenix dactylifera* myb-binding protein 1A-like protein (LOC103719001), mRNA	103719001	NA	100
$PdT_{11}A/AP7-6B$	1361	XM_008783430.1	Predicted: *Phoenix dactylifera* mitogen-activated protein kinase kinase kinase YODA-like (LOC103701392), mRNA	103698668	(pda04016) MAPK signaling pathway-plant.	87
$PdT_{11}A/AP7-7B$	942	XM_008804820.1	Predicted: *Phoenix dactylifera* bifunctional UDP-glucose 4-epimerase and UDP-xylose 4-epimerase 1-like (LOC103716713), transcript variant X2, mRNA	103716713	(pda00052) Galactose metabolism (pda00520) Amino sugar and nucleotide sugar metabolism (pda01100) Metabolic pathways	91
$PdT_{11}A/AP1-1M$	1091	XM_008791058.2	Predicted: *Phoenix dactylifera* NF-X1-type zinc finger protein NFXL1 (LOC103706810), mRNA.0	103706810	NA	92
$PdT_{11}A/AP7-2M$	1175	XP_008779208.1	Predicted: Ent-copalyl diphosphate synthase 1, chloroplastic-like, partial [*Phoenix dactylifera*]	----	Unknown	32
$PdT_{11}A/AP7-3M$	1244	XM_008802998.1	Predicted: *Phoenix dactylifera* putative magnesium transporter MRS2-G (LOC103715388), mRNA	103715388	NA	92
$PdT_{11}A/AP7-4M$	373		No significant similarity found			
$PdT_{11}A/AP7-5M$	1282	XM_008809320.2	Predicted: *Phoenix dactylifera* cyclin-dependent kinase G-2 (LOC103719868), transcript variant X10, mRNA	103719868	NA	81
$PdT_{11}A/AP7-6M$	864	XP_008778003.1	Predicted: ABC transporter C family member 13-like isoform X2 [*Phoenix dactylifera*]	103717193	(pda02010) ABC transporters	42
$PdT_{11}A/AP7-7M$	1045	XP_017701444.1	Predicted: probable indole-3-pyruvate monooxygenase YUCCA1 [*Phoenix dactylifera*]	103720204	(pda00380) Tryptophan metabolism (pda01100) Metabolic pathways	36
$PdT_{11}A/AP7-8M$	1148	XM_008795569.2	Predicted: *Phoenix dactylifera* ATPase family AAA domain-containing protein 1-A (LOC103710008), transcript variant X2, mRNA	103707726	NA	100
$PdT_{11}A/AP7-9M$	1073	XM_008800687.2	Predicted: *Phoenix dactylifera* transcription factor bHLH74-like (LOC103713676), transcript variant X2, mRNA	103704278	NA	92
$PdT_{11}A/AP7-10M$	754	XM_008784799.2	Predicted: *Phoenix dactylifera* phosphoglycolate phosphatase 2 (LOC103702382), mRNA	103702382	(pda00630) Glyoxylate and dicarboxylate metabolism (pda01100) Metabolic pathways (pda01110) Biosynthesis of secondary metabolites (pda01200) Carbon metabolism	92

NA: Not available

membrane by Reactive Oxygen Species (ROS). Lipid peroxidation correlates with MDA concentration[11]. Lowered MDA production observed in the Bertamoda against highly accumulation of MDA in Malkabi cultivar suggests that Bertamoda is relatively higher tolerance to salinity stress than Malkabi, which can be due to the presence of higher free radical-scavenging capacity and protection mechanisms in the Bertamoda. This was found to be consistent with works done by Suhim *et al.*[32].

Salt treatment with NaCl decreased significantly chlorophyll a and total chlorophyll content (Table 2)[33-35]. This may be due to ion accumulation, functional disturbance and rapid maturing of leaves under the effect of salinity stress[36]. It is observed that the chlorophyll content decreased in salt sensitive genotypes in comparison to tolerant ones[34,37].

Excess Na^+ accumulation in plants under salt stress often induce oxidative stress through the production of ROS[14,37]. The ROS degrade membrane lipids resulted in leakage of potassium ions[38,39]. Assessing Na^+, K^+ and K^+/Na^+ considered as a main indicator to salt tolerance in plants, where maintaining a high K^+/Na^+ correlates with salt tolerance and severe increase in Na^+ associated with severe decrease in K^+ correlates with plant's susceptibility to salt stress[40]. The up-regulated ESTs in response to salt stress conditions were identified for both cultivars Bertamoda and Malkabi (Table 4). Inositol family is very important for plant adaptation to salt stress[41,42]. Also, inositol is an important precursor of the Raffinose Family Oligosaccharides (RFOs) that accumulate in plants under abiotic stresses[43] and protecting cellular structures from ROS by controlling turgor pressure[44]. Salt tolerant plants tend to accumulate sodium in the vacuole. In *Arabidopsis*, over expression of Proton Pumping Pyrophosphatase type I (H^+-PPase AVP1) resulted in enhanced salinity and drought tolerance[45,46]. ATPases in general are well-known to translocate various ions across membranes including Ca_{2+}, H^+ and Na^+/K^+. In *Arabidopsis thaliana*, Phospholipid-transporting ATPase 3 was found to change membranes through changes of structural or functions enabling the plant to grow and tolerate different abiotic stresses[47].

Mitogen-Activated Protein Kinase (MAPK) involved in significant operations, such as, hormone responses and plant immunity[36]. Plant MAPK regulate plant development and defense under sever conditions[48]. According to Li *et al.*[49], defense response related genes were up-regulated by MAPKKK4 expression on rape seed. Abiotic stresses have an impact on carbohydrates correlating with cell membrane and cell wall fluidity and integrity[50]. Adjusting cell wall elasticity is important to maintain turgor pressure and water uptake[51]. In this study, UDP-glucose-4-epimerase (UGE) enzyme was up regulated, that catalyses the reversible conversion of UDP-glucose to UDP-galactose. Galactose is a precursor for xyloglucan which is a hemicellulose component implicated in cell wall biosynthesis during cell expansion[52]. Gibberellins (GAs) are phytohormones that regulate many plant growth processes, such as germination and stem elongation[53,54]. GA concentration are directly prolonged with the rate of *ent*-kaurene production[55]. Ent-CDP synthase is a precursor to phytoalexins that known as defensive compounds produced by the plant[56]. Cyclin-Dependent Kinase G2 (CDKG2) act as a negative regulator of the salt stress response. Ma *et al.*[57] observed that the loss of CDKG2 function decreased the severity of salt stress. The kinase activity associated with CDKs requires the binding of a regulatory cyclin partner to be activated[58-60].

It was observed on this study, expression of CDKG2 in Malkabi cultivar may indicate it's less tolerant to salt stress than Bertamoda cultivar. YUC1 involved during embryogenesis and seedling development of plant It belongs to the set of many *YUCCA* (YUC) genes responsible for auxin biosynthesis[61] that are mainly expressed in meristematic, vascular tissues and reproductive organs[62]. The observation that YUC1 over-expression is lined with high levels of IAN and IAA production[63]. IAA is important for normal plant development and growth[64]. Under stress conditions the up-regulation of such pathways in this study through the *YUC1* gene is an attempt to resist salinity and keep the plant in a viable state. Adenosine triphosphatase (ATPase) belong to the AAA protein family that involved in many activities such as protein folding, transcription control and in abiotic stress tolerance[65,66]. The exposure of plant to abiotic stress such as salinity stress reduced the photosynthetic activity and components of the electron transport system because the productions of Phosphoglycolate Phosphatases[67]. The MYB family members are found to be involved in regulating plant responses to various abiotic and biotic stresses[68,69]. Several studies have confirmed that MYB TFs play important roles in adaptation to several different environmental stresses and trigger the expression of stress-induced genes that are essential for plants to tolerate abiotic stresses especially drought and salt stress[70-72] At *NFXL1* gene encodes NF-X1 that plays a key role in salt tolerance[73], improves plant growth and physiological mechanisms under stress. Transcription factor *bHLH74* encodes a basic helix-loop-helix (bHLH) transcription

factor[74]. It was predicted that this family have roles in plant metabolism and tissue development[75]. Bahieldin *et al.*[76] stated that detect putative universal stress proteins (USPs) in RNA-Seq of the *Catharanthus roseus.* Several of these TF super-families are help the plant to defense against abiotic and biotic stresses by affects downstream genes functioning at a particular time on target cells. It can also be responsible for the direction of the transcriptional activity of a target gene[77]. Different families of putative magnesium transporters MRS2-G have been proved to be correlated to salt stress in different plants[78]. Up-regulation of the gene in this study suggested that active involvement in transporting ions across membranes in response to salinity stress. The role of ABC transporter proteins among plants is detoxification in which they are required for plant development, resistance to pathogen and the interaction of the plant with abiotic stresses[79]. To achieve these roles, it is depositing surface lipids and transporting the phytohormones like abscisic acid and auxin[80]. The ESTs with no significant sequence similarity to known sequences in the genbank need more investigation to confirm the relation to the abiotic stress process or may represent yet uncharacterized gene.

CONCLUSION

This study concluded that Bertamoda cultivar has the ability to tolerate salt stress than Malkabi cultivar because the ability of this cultivar to protect the photosynthetic pigments, absorb low amounts of Na^+ and minimize the production of MDA as a response of salt stress. Also, by up regulation of 17 genes which are controlling the expression of many metabolic pathways that have a role in protecting cellular structures and improving cellular functions enabling the plant to grow and keep it in a viable state and tolerate salinity.

SIGNIFICANCE STATEMENT

This study will help the researcher to understand the mechanisms that date palm cultivar take to tolerate salt stress and breeders to improve Date palm breeding programs also, these up-regulated genes can be transferred to another plant to give them the power to tolerate salinity.

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