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Using cDNA SCoT method to identify differentially expressed genes in date palm (*Phoenix dactylifera* L.)

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Research Article

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Abstract

Date palm is a relatively salt- and drought-tolerant plant and more recent efforts have been focused on recognizing genes and pathways that confer stress tolerance in this species. The cDNA start codon targeted marker (cDNA–SCoT) technique is a novel, simple, fast and effective method for differential gene expression investigation. In the present study, this technique was exploited to identify differentially expressed genes during several stress treatments in date palm. A total of 12 SCoT primers combined with oligo-dT primers amplified differentially expressed fragments among the stress treatments and control samples. Differentially expressed fragments were highly homologous to known genes or encoded unclassified proteins with unknown functions. The expression patterns of the genes that had direct or indirect relationships with salinity and drought stress response were identified and their possible roles were discussed. This study suggests that the cDNA-SCoT differential display method is a useful tool to serve as an initial step for characterizing transcriptional changes induced by abiotic stresses and provide gene information for further study and application in genetic improvement and breeding in date palm.

Introduction

Date palm (*Phoenix dactylifera* L.), belonging to the *Arecaceae* family, is the main crop of the arid region of western Asia and North Africa (Munier, 1973; Barrow, 1998; Zohary *et al*., 2012). It is the key species in the palm family, which consist of about 200 genera and more than 2500 species (El Hadrami and El Hadrami, 2009; Jain *et al*., 2011). Throughout the previous three centuries, dates were introduced to Mexico, India, Pakistan, southern Africa, Australia and United States (Zohary *et al*., 2012). It represents about 1,235,601 ha and a living revenue of 10 million oases worldwide. In the oasis, date palm, just like other plants, is routinely exposed to an unpredictable combination of diverse stresses (Slama *et al*., 2015), which is even worse in the circumstances of climate change, soil salinization and environmental pollution. Plants commonly encounter severe growing conditions like low or high temperature, deficient or excessive water, high salinity, heavy metals and ultraviolet radiation (He *et al*., 2018). These abiotic stresses can affect plant growth, causes irreversible damage and drives to death. They represent the main causes of crop yield penalty worldwide (Bray *et al*., 2000; Agarwal and Jha, 2010).

Plants have to get around the stresses and develop powerful adaptive strategies to tolerate their adverse effects allowing them to survive and thrive. Therefore, plants are able to adapt their metabolism to diverse fluctuations in the environment throughout evolution (Maksymiec, 2007). Plant stress responses are dynamic and implicate cooperation amongst diverse regulatory levels, comprising modification of metabolism and gene expression for physiological and morphological adaptations; for instance, stress protein production, antioxidant up-regulation and compatible solute accumulation (Xiong *et al*., 2002; Krasensky and Jonak, 2011). Plant species vary in their tolerance to abiotic stress. Salt stress causes more than 50% yield losses in major crops around the world depending on the crop (Al-taweel *et al*., 2019) and affects the many metabolic and physiological processes of the plant, which can damage the cells.

Molecular techniques have been proposed to be appropriate powerful tools for the identification of some clonal variation, stress tolerance and establish genetic stability (Bennici *et al*., 2004; Khaled *et al*., 2018). In recent times, many new promising alternative marker techniques, refining developments in the field of molecular biology, have developed. In this context, numerous approaches have been developed for the analysis of differential gene expression at the mRNA level in various plants. Recently, a new marker has been developed for the assessment of genetic diversity, called start codon targeted (SCoT) marker (Collard and Mackill, 2009). The start codon (ATG) and flanking sequences are highly conserved in the plant

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genes (Sawant *et al.*, 1999). In SCoT marker profiling, a single primer is used as in ISSR and RAPD markers. Conversely, this marker is more reproducible than the RAPD as it has a longer primer sequence (Mulpuri *et al.*, 2013). The SCoT marker has been used for the assessment of genetic diversity in *P. dactylifera* cultivars and gave reproducible results among the cultivars (Al-Qurainy *et al.*, 2015; Rhouma *et al.*, 2020). SCoT markers can be developed from the transcribed regions and might be related to the gene function as proven in mango (*Mangifera indica* L.) (Luo *et al.*, 2014) and sugarcane (*Saccharum officinarum*) (Wu *et al.*, 2013).

The cDNA-SCoT method is a new gene expression analysis method developed by Wu *et al.* (2013), compared with all other differential gene analysis methods available in the current research fields. It is relatively more efficient, rapid, inexpensive and simple to operate. Its data results are also easily reproducible (Luo *et al.*, 2014). For instance, even though the cDNA-AFLP and cDNA-SRAP methods are also reliable methods for gene expression analysis, the cDNA-SCoT method is much simpler since it needs fewer primer combinations to operate. This technique will certainly play an essential role in studying differentially expressed genes, finding new genes and exploring the molecular mechanism of resistance (Wu *et al.*, 2013).

The optimal environment is extremely variable for plant species. Harsh environments may be damaging for one plant species, however may possibly not be for another (Munns and Tester, 2008). These facts designate that plant response to the environment are complex and that specific sensitivities exist throughout the multitude of plant species. Knowledge of molecular mechanisms in *P. dactylifera* L. under abiotic stress conditions is limited. Yaish and Kumar (2015) suggest that *P. dactylifera* L. can acclimate to extreme drought, heat and moderately elevate the stages of soil salinity. Therefore, in the present study, the experiments were carried out using seedlings from the elite cultivar 'Deglet Noor' to determine the performance for growth and yield-related traits and gene expression profiling under different stress levels.

Material and methods

Plant material, growth and experimental design

Seeds of 'Deglet Noor' cultivar were collected from the oasis of Tozeur in the South of Tunisia. The healthy seeds of *P. dactylifera* were cleaned, scraped, soaked for 48 h in lukewarm water and brushed to remove residues. Surfaces were sterilized with 75% ethanol for 3 min and then with sodium hypochlorite solution (12°) for 10 min and washed thoroughly four times with distilled water. Subsequently, the seeds were removed into sterilized wet Petri dishes and incubated at 37°C until germination, which was then transplanted into 2 litre pots. The pots were filled with a mixture of sand and peat moss (1:1). The sand was sieved, washed successively with tap water and rinsed with distilled water. The seeds were sown in plastic pots and watered at regular intervals to maintain moisture for better germination. The pots were maintained in the growth chamber at 26–27 °C, daylight and relative humidity of 72%.

The stress conditions were salt stress using 240 mM NaCl (four samples), salt stress using 50 mM $CaSO_4$ (four samples), drought using polyethylene glycol (PEG, MW 6000) 82.5 g/l (four samples) and drought due to no irrigation (four samples). The experiment was carried out for 3 weeks along with four control samples (irrigated with distilled water) (Fig. 1). Leaves and roots were collected after each treatment and stored at −80°C for RNA isolation

Biomass and morphological traits

Fresh leaf and root weight and shoot and root length were measured after 3 weeks of treatment. Each treatment was compared to control plants for the evaluation of their stress responses. The statistical analysis of the morphometric parameters was carried out using the software program IBM SPSS Statistics 20. The difference in variance between the treated groups was compared by an ANOVA test. The difference is significant at a p-value ≤ 0.05.

RNA extraction and differential gene expression analysis

Total RNA was extracted from the control and stressed plants using the GF-1 Total RNA Extraction Kit (Vivantis, Malaysia) according to the directives specified in the manual. The quantity and quality were verified using the spectrophotometer (Gold S54T, Shanghai). High-quality cDNA was prepared using the PrimeScript™ 1st strand cDNA Synthesis Kit (TaKaRa). The PCR reaction was performed in a total volume of 25 µl using the SCoT primers (Table 1) for the study of expression profiling.

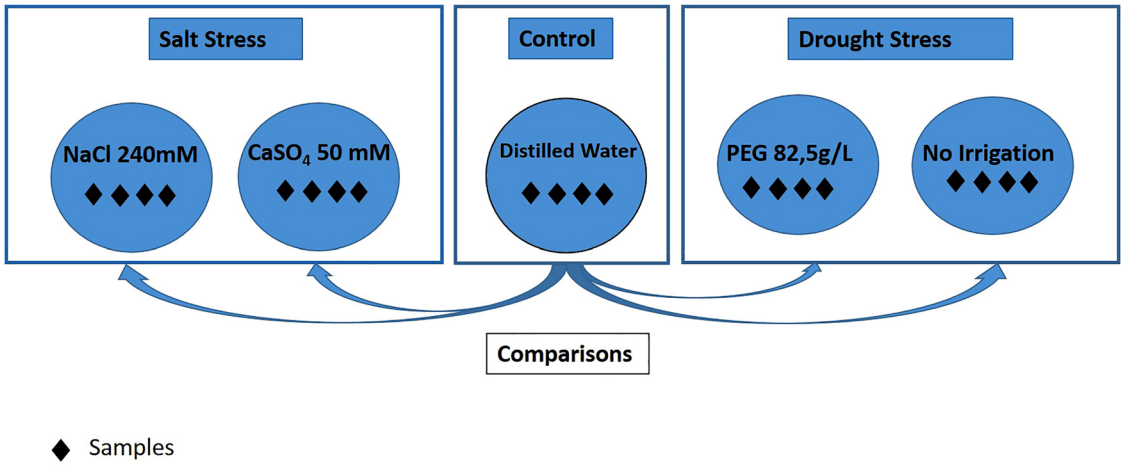


Fig. 1. Different treatments on date palm seedlings in advance of transcriptomic analysis.

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These primers were selected for their effectiveness in revealing polymorphism in date palms (Rhouma *et al.*, 2020). The cDNA was diluted in RNase-free water to a working concentration of 50 ng for PCR amplification with SCoT primer (20 picomoles per reaction). PCR was performed in a 2720 thermal cycler (Applied Biosystems). The cycling profile was 94°C for 3 min, 45 cycles at 94°C for 1 min, 44.5°C for 30 s, 72°C for 1 min and a cycle of 72°C for 5 min. The amplified products were resolved on 2% TBE agarose gel. The oligo-dT anchored cDNA-SCoT patterns were compared for each stress treatment and control.

Isolation and sequencing of oligo-dT anchored cDNA-SCoT fragments

According to the results of the amplification, differential fragments were found based on their absence or differential intensity on an agarose gel, and then the polymorphic expressed sequence tags were cut from the agarose gel with a sharp scalpel. The target products were purified by DNA extraction using an agarose gel kit (EZ-10 Spin Column DNA Gel Extraction Kit, Bio Basic) and sequenced. The sequences were analysed for their homology with nucleotide sequences through BLASTX searches against the GenBank non-redundant public sequence database (http://www.ncbi.nlm.nih.gov/BLAST).

Results and discussion

Effect of stress on morphometric parameters:

In order to study the effect of salinity and drought on the morphometric parameters, of treated and control seedlings, we measured the length and weight of the root and aerial parts. The measures were carried out immediately after the date palm seedlings were removed from the culture bags and washed (Fig. 2). Statistical analysis showed no significant difference in morphological parameters (mass and length) (Fig. 3). Indeed, the absence of morphological differences between treated and untreated seedlings may be related to the short duration of stress and/or by the capacity of adaptation to drought and salinity of *P. dactylifera* which is superior to other plant species.

Table 1. SCoT primers used for PRC reactions

No.	Primers code	Sequence 5′-3′	%GC
1	SCoT 10	CAACAATGGCTACCAGCC	55.6
2	SCoT 45	ACAATGGCTACCACTGAC	50
3	SCoT 41	CAATGGCTACCACTGACA	50
4	SCoT 5	CAACAATGGCTACCACGA	50
5	SCoT 7	CAACAATGGCTACCACGG	55.6
6	SCoT 9	CAACAATGGCTACCAGCA	50
7	SCoT 23	CACCATGGCTACCACCAG	61.1
8	SCoT 26	ACCATGGCTACCACCGTC	61.1
9	SCoT 30	CCATGGCTACCACCGGCG	72.2
10	SCoT 35	CATGGCTACCACCGGCCC	72.2
11	SCoT 42	CAATGGCTACCATTAGCG	50
12	SCoT 44	CAATGGCTACCATTAGCC	50

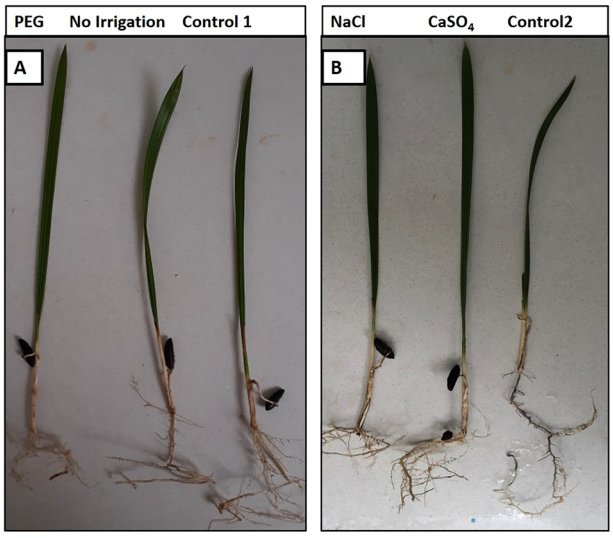


Fig. 2. Seedling after 20 days of stress. (A) Drought stress; (B) saline stress.

In fact, some cultivars of date palms have the capacity to grow close to the seashore where they are often exposed to seawater during tidal currents. Therefore, the date palm could be considered an exceptional halophyte plant and could possess a series of mechanisms of tolerance to salinity. Our results are in agreement with those described by Yiash *et al.* in 2017 where the cultivar Khalas subjected to severe salt stress for 10 days did not show morphological differences compared to the control seedlings. Moreover, Al Karusi *et al.* (2019) demonstrate that a saline treatment for a longer period (6 weeks) showed a 20 and 27% reduction in leaf area relative to the cultivars Umsila and Zabad and a decrease in root length and surface area. Other studies have shown that, under saline stress, other symptoms were visualized such as leaf tip burning (Al Kharusi *et al.*, 2017).

On the other hand, it has been described that, under drought stress conditions, date palm develops several adaptation mechanisms including morphological changes in the aerial and/or root part. In 2016, Elshibli *et al.* (2016) showed that a date palm seedling displays multiple transformations in growth and morphology under drought-stressed conditions of 10 and 25% of plant capacity. In addition, a study of two cultivars developed *in vitro* under PEG-induced water stress allowed the selection of the most drought-tolerant cultivar (Djibril *et al.*, 2005). The response to water stress is genotype-specific, as it is possible to identify and select the cultivars best adapted to improve yield in the most sensitive areas. Other studies carried out on date palm in open fields have shown the effect of water stress on different morphological parameters. Actually, Gribaa *et al.* (2013) and Sabri *et al.* (2017) reported a negative effect on date production in quantity and quality, in addition to the adverse effect on the aerial and root part.

Isolation and analyses of transcript-derived fragments (TDF) and function predictions

Analysis of gene expression is a central aim in most studies of molecular and cellular biology. It forms the basis for understanding plant growth, development and adaptation and permits the identification of precise regulator points of metabolism (Gupta and Chakrabarty, 2013). In this context, Oligo-dT cDNA-SCoT

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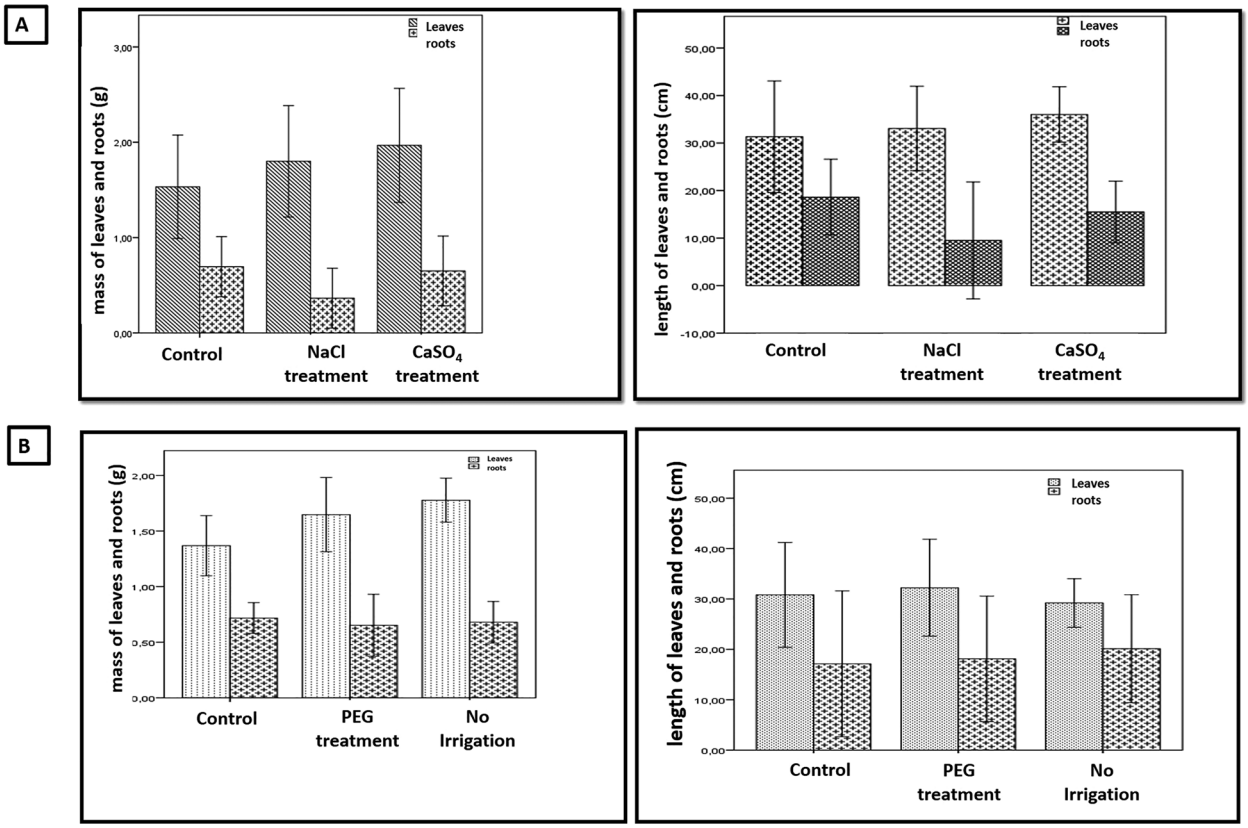


Fig. 3. Fresh leaves and root weight and length in *Phoenix dactylifera* L. grown in the green house at different saline (A) and drought (B) treatments. Data represent means of four replicates ± standard deviation.

analysis was performed on RNA isolated from leaves and roots collected to identify genes response to stress treatments in *P. dactylifera*. A total of 12 SCoT primers were used for PCR amplification. SCoT polymorphism is a gene-targeted marker that can generate information correlated with biological traits compared with random DNA markers (Collard and Mackill, 2009; Xiong *et al.*, 2009). Only those with stable amplification results and clear bands were selected. By using these 12 single primers, 108 cDNA fragments with the length of 100–3000 bp were amplified. Results show qualitative (presence or absence of bands) and quantitative differences (variability in band intensity). These results prove the effectiveness of the cDNA-SCoT technique. In fact, Al-Qurainy *et al.* (2017) have shown its efficacy in the study of differential expression between date palm plants under salt stress induced by various concentrations of NaCl. In addition, this technique has been used to study genes expressed at different stages of development of sugar cane seedlings treated or not with gibberellic acid (Wu *et al.*, 2013) and results showed that a SCoT primer could produce three to 15 differentially expressed bands. Moreover, cDNA SCoT was equally used to study gene change and to understand the allopolyploidy evolutionism and the genetic mechanism of Arachis interspecific hybridization (He *et al.*, 2017). Due to high success rate, low false-positive rate, simple operation and low cost, cDNA-SCoT techniques were appropriate for analysing the variations of gene expression in those different plant species.

Thirty-three clear, bright and differentially expressed bands were selected and sequenced. After verification of the sequences obtained, it results that some of them do not present any significant homology. On the other hand, others present homologies with certain species and whose presence may be strongly associated with the stress applied. All of these sequences, their homologies and their possible functions are summarized in Table 2. TDFs sharing high homology with genes in the NCBI database could be divided into four types according to their functions, including resistance-related genes, signal transduction factor, transcription and translation factor-related genes and unknown functional protein-related genes. TDFs, expressed under drought or saline stress, are considered as not significant since they have unknown functional protein-related or are not associated to either biotic or abiotic stress tolerance.

Features of genes differentially expressed during drought stress

Analysis of differentially expressed sequences, due to drought stress, showed homology with genes whose expression could be strongly associated with the applied treatment (Table 2). In fact, TDF1, expressed within the PEG-stressed stem, has homology with the sequence of *P. dactylifera* L. which codes for the protein DNA ligase 1-like controlling the repair of broken DNA following high levels of environmental stress (Waterworth *et al.*, 2009). The expression of this gene indicates its role in stress response, as effective cellular response mechanisms have evolved to cope with DNA damage caused by water stress, including delay or arrest of the cell cycle and activation of repair pathways and DNA ligase ligation. TDF2, a fragment expressed at the PEG-stressed stem, has homology with the sequence of *P. dactylifera*. L, which codes for the eukaryotic translation initiation factor 3. This factor, described in *Arabidopsis thaliana*, is responsible

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Table 2. Function prediction of transcript-derived fragments (TDFs) by the cDNA start codon targeted polymorphism (cDNA-SCoT) technique

	Origin of TDFs	Homological gene	Accession number	Functional category
TDF1	PEG-stressed stem	DNA ligase1-like	XM_008792098.2	Reparation and ligation of DNA strand breaks
TDF2	PEG-stressed stem	Eukaryotic translation initiation factor 3	XM_008786463.3	Initiation of the translation of a subset of mRNA involved in cell proliferation
TDF3	Absence of irrigation	Serine/arginine repetitive matrix protein 2 (*Phoenix dactylifera* L)		Splicing factors; regulation of alternative splicing
TDF4	Absence of irrigation	Late embryogenesis abundant proteins (LEA)	CP046694.1	Involved in the response of plants to abiotic stress
TDF5	NaCl-stressed stem	Nucléolin 1-like (*Phoenix dactylifera* L.)	XM_026802420.1	Multifunctional nuclear phosphoprotein (Tuteja and Tuteja, 1998) involved in adaptation to salt stress (Boonchai *et al*., 2018) and signal transduction
TDF6 (2)	NaCl-stressed stem – absence of irrigation	Serine/arginine repetitive matrix protein 2 (*Phoenix dactylifera* L)	XM_008778408.3	Splicing factors; regulation of alternative splicing
TDF7	NaCl-stressed roots	S-related kinase (*Arabidopsis lyrata*)	AY186754.1	Protein kinase involved in ABA signalling pathways
TDF8	NaCl-stressed roots	Element-binding protein 2-like (*Phoenix dactylifera* L.)	XM_008797243.3	Transcription factor
TDF9	CaSO4-stressed stem	Homeobox-leucine zipper protein HAT5 (*Capsicum annuum*)	XM_016692739.1	Transcription factor linked to DNA and an adjacent leucine zipper motif (Henriksson *et al*., 2005)
TDF10–TDF33	Drought/saline stress	Unknown functional protein-related genes	–	–

for initiating the translation of a subset of mRNAs involved in cell proliferation. Stress has adverse effects on the growth of the seedling, consequently on cell proliferation. The expression of this factor aims to enhance cell proliferation in order to overcome water stress. Moreover, TDF3 which is expressed at the level of the stressed stem, due to the absence of irrigation, has a homology

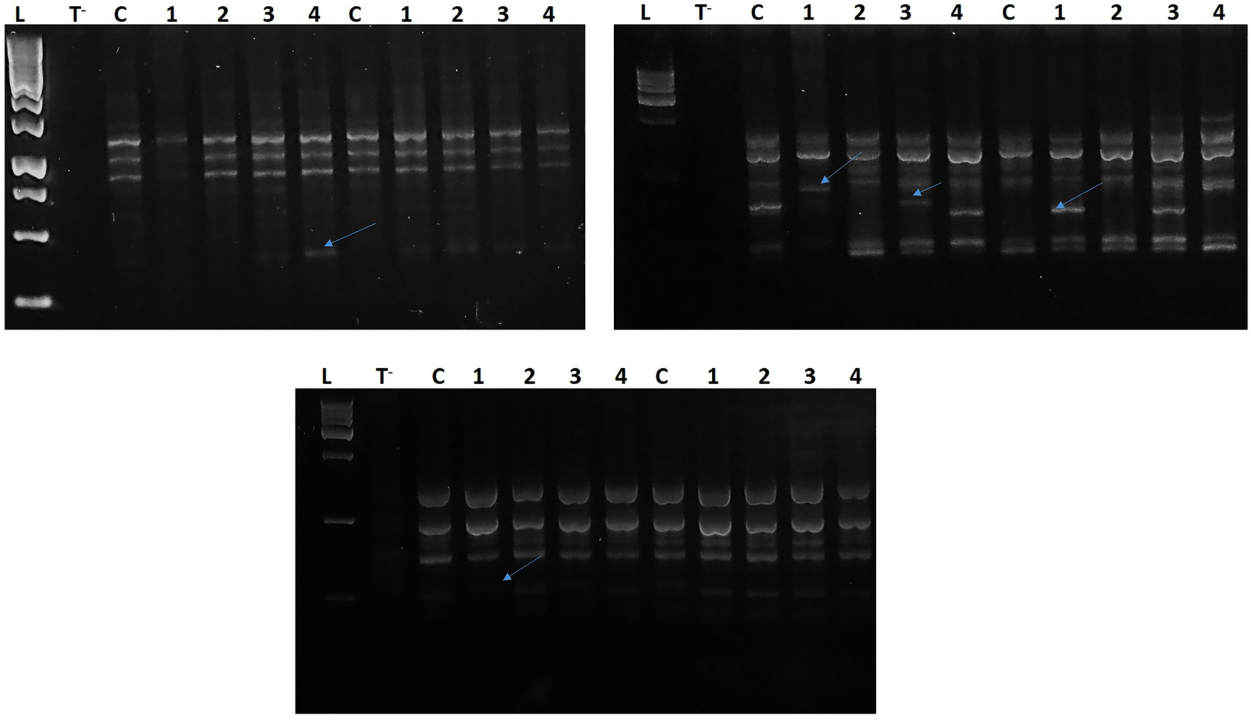


Fig. 4. cDNA-SCoT marker profiling generated from individual plant leaves of Phoenix dactylifera L. at different stress treatments. Lane L: 100 bp ladder; Lane T-: negative control of PCR, lane C: control (seedling without treatment); lanes 1, 2, 3, and 4: seedling with drought or saline treatment. The arrows indicate some differentially expressed bands.

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with the sequence of *P. dactylifera* L. which codes for a repetitive serine/arginine matrix protein 2. The latter interacts with other factors involved in messenger RNA maturation to activate the alternative splicing of numerous genes encoding factors involved in signal transduction, cell cycle and transcription (Yoshimura *et al.*, 2011). These results suggest the existence of an important transcriptome diversification under the effect of water stress. Finally, Chen *et al.* (2019) showed that late embryogenesis abundant protein (LEA) is a large and highly diverse family, and it plays a role in normal plant growth and development and protects cells from abiotic stress (drought, salt, heavy metals, heat and cold). The genes that code for LEA proteins are located in clusters on chromosomes 1, 2 and 10 of the *Solanum tuberosum* plant. In our study, TDF4 shows a similarity with chromosome 10 of *Solanum tuberosum*; we can deduce that our sequence corresponds to a gene coding for one of the LEA proteins involved in the response to water stress applied to the date palm seedling.

Features of genes differently expressed during saline stress

Plant salt tolerance depends on internal osmotic adjustment phenomena to maintain water with tissue turgidity. Depending on their response to saline conditions, plants can be divided into two types: (1) halophytic plants that tolerate high concentrations of salt (this tolerance is due to biochemical and molecular adaptation mechanisms mainly the compartmentalization of salt in the vacuole (Parida and Das, 2005)); and (2) glycophytic plants that are sensitive to high salt concentrations. These concentrations cause ionic toxicity and osmotic stress until the death of these plants.

Numerous regulatory genes are involved in different metabolic pathways of date palm exposed to saline stress (Table 2). In fact, analysis of differentially expressed genes under saline stress showed variability in the transcript-derived fragments. Indeed, TDF5 amplified by the SCoT 41 primer differentially expressed at the stem treated with NaCl shows homology with the gene encoding the Nucléolin 1-like protein (79.58%). This protein is multifunctional and is located mainly in the nucleoli, nucleoplasm, cytoplasm and cell membrane. It is involved in cell proliferation, ribosome biogenesis (rRNA synthesis), chromatin stability, DNA and RNA metabolism, stress response and signal transduction (Jia *et al.*, 2017). Nucleolin overexpression has enabled the adaptation of transgenic rice to saline stress (Boonchai *et al.*, 2018). Differential expression of this structural gene, coding for a regulatory protein, in the stressed date palm may confer tolerance to salinity. TDF6, amplified by the SCoT 41 primer on the NaCl-treated stem, has homology to a predicted sequence of serine/arginine repetitive matrix protein expressed in *P. dactylifera* L. This protein is involved in the regulation of alternative splicing and transcriptome diversity in *Arabidopsis* in response to high light stress (Yoshimura *et al.*, 2011) which could be a key gene involved in the tolerance mechanism of date palm to saline stress. Moreover, TDF7 is homologous to the S-related kinase gene, actually observed in *Arabidopsis lyrata*. It is well known that kinase proteins are rapidly activated by osmotic stress and are involved in ABA signalling pathways (Lin *et al.*, 2020). The expressed sequence confirms the role of these proteins in the osmoregulation of the date palm to maintain its growth in the presence of environmental constraints. TDF8, expressed differentially in roots stressed by NaCl, corresponds to an element-binding protein 2-like sequence observed in *P. dactylifera* L. These elements are transcription factors allowing the activation of genes sensitive to heat stress and drought, which induces a high tolerance to these constraints in *A. thaliana* (Mizoi *et al.*, 2019). The expression of these elements in date palm increases the tolerance of the latter to saline stress. Finally, TDF9 gene is expressed in stems stressed by CaSO4. This gene is identical to the predicted *Capsicum annum* sequence of homeobox-leucine zipper protein HAT5 (transcription factors unique to the plant species) involved in plant growth, regulation of responses to biotic and abiotic stresses, and modulation of hormone action (Ribone *et al.*, 2016). The expression of this gene in date palm allows its adaptation to CaSO4-induced stress.

Conclusion

Date palm is one of the most popular fruit crops in arid, semiarid, tropical and subtropical regions. It generally grows under hostile climatic circumstances and has therefore developed stress tolerance throughout its evolution. It is known to subsist under drastic drought, heat and high soil salinity rate (Yaish and Kumar, 2015). Adaptation to these conditions includes evolutionary consequences detectable in a wide range of morphological and molecular multifunctional responses (He *et al.*, 2018). Genomic studies of the date palm over the last decade have led to the identification of several key genes associated with fruit colour and sugar accumulation (Hazzouri *et al.*, 2019). However, there is little understanding of the genetic aspects underlying different biotic and abiotic stresses. Analysis of stress-induced differential gene expression can provide valuable information on the adaptive mechanisms of date palms.

Our results showed that rapid and considerable gene expression variations began as early as abiotic stress threatened the date palm crop. Several types of gene expression changes were observed, including quantitative and qualitative (novel gene expression) variations. The cDNA-SCoT technique permitted us to successfully investigate the variations of gene expression in date palms. This study provides robust candidate genes for future functional research and a basis for improving abiotic stress tolerance in *P. dactylifera* crop. Indeed, genome-wide investigation of the structural diversity, phylogenetic relationships and functional attributes of those gene families is required. Furthermore, the expression of selected genes through quantitative real-time PCR will provide new insights into understanding the mechanism of salt and drought tolerance in date palm species.

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Conflict of interest. None.

References

Agarwal PK and Jha B (2010) Transcription factors in plants and ABA dependent and independent abiotic stress signaling. *Biologia Plantarum* **54**, 201–212.

Al-Qurainy F, Khan S, Nadeem M and Tarroum M (2015) SCoT marker for the assessment of genetic diversity in Saudi Arabian date palm cultivars. *Pakistan Journal of Botany* **47**, 637–643.

Al-Qurainy F, Khan S, Nadeem M, Tarroum M and Gaafar ARZ (2017) Antioxidant system response and cDNA-SCoT marker profiling in *Phoenix dactylifera* L. plant under salinity stress. *Integrative Journal of Genomics*, Article ID 1537538, 10 pages. https://doi.org/10.1155/2017/1537538.

Al-taweel SK, Abdel-Aziz RM, Rabea K and Khaled K (2019) Studying cDNA SCoT in response to salinity stress in Stevia Rebaudiana Bertoni. *Journal of Breeding Genetics* **51**, 281–294.

https://doi.org/10.1017/S1479262123000175 Published online by Cambridge University Press

Al Kharusi L, Assaha D, Al-Yahyai R and Yaish M (2017) Screening of date palm (*Phoenix dactylifera* L.) cultivars for salinity tolerance. *Forests* **4**, 136.

Al Kharusi L, Sunkar R, Al-Yahyai R and Yaish MW (2019) Comparative water relations of two contrasting date palm genotypes under salinity. *International Journal of Agronomy*. Article ID 4262013, 16 pages. https://doi.org/10.1155/2019/4262013.

Barrow S (1998) A monograph of Phoenix L. (Palmae: Coryphoideae). *Kew Bulletin* **53**, 513–575.

Bennici A, Anzidei M and Vendramin G (2004) Genetic stability and uniformity of *Foeniculum vulgare* Mill. Regenerated plants through organogenesis and somatic embryogenesis. *Plant Science* **166**, 221–227.

Boonchai C, Udomchalothorn T, Sripinyowanich S, Comai L, Buaboocha T and Chadchawan S (2018) Rice overexpressing OsNUC1-S reveals differential gene expression leading to yield loss reduction after salt stress at the booting stage. *International Journal of Molecular Sciences* **19**, 3936.

Bray EA, Bailey-Serres J and Weretilnyk E (2000) Responses to abiotic stresses. In Gruissem W, Buchannan B, Jones R (eds), *Biochemistry and Molecular Biology of Plants*. Rockville, MD: American Society of Plant Physiologists, pp. 1158–1249.

Chen Y, Li C, Zhang B, Yi J, Yang Y, *et al.* (2019) The role of the late embryogenesis-abundant (LEA) protein family in development and the abiotic stress response: a comprehensive expression analysis of potato (*Solanum tuberosum*). *Genes* **10**, 148.

Collard BC and Mackill DJ (2009) Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter* **27**, 86.

Djibril S, Mohamed OK, Diaga D, Diégane D, Abaye B F, *et al.* (2005) Growth and development of date palm (*Phoenix dactylifera* L.) seedlings under drought and salinity stresses. *African Journal of Biotechnology* **4**, 968–972.

El Hadrami I and El Hadrami A (2009) Breeding date palm. In Ja SM and Priyadarshan PM (eds), *Breeding Plantation Tree Crops*. New York: Springer, pp. 191–216.

Elshibli S, Elshibli E M and Korpelainen H (2016) Growth and photosynthetic CO2 responses of date palm plants to water availability. *Emirates Journal of Food and Agriculture* **28**, 58–65.

Gribaa A, Dardelle F, Lehner A, Rihouey C, Burel C *et al.* (2013) Effect of water deficit on the cell wall of the date palm (*Phoenix dactylifera* 'Deglet nour', Arecales) fruit during development. *Plant Cell Environment* **36**, 1056–1070.

Gupta R and Chakrabarty SK (2013) Gibberellic acid in plant: still a mystery unresolved. *Plant Signaling & Behavior* **8**, e25504. https://doi.org/10.4161/psb.25504. PMID: 23857350; PMCID: PMC4002599.

Hazzouri KM, Gros-Balthazard M, Flowers JM *et al.* (2019) Genome-wide association mapping of date palm fruit traits. *Nature Communications* **10**, 4680.

He M, He C-Q and Ding N-Z (2018) Abiotic stresses: general defenses of land plants and chances for engineering multistress tolerance. *Frontiers in Plant Science* **9**, 1771.

Henriksson E, Olsson AS, Johannesson H, Johansson H, Hanson J, Engström P and Söderman E (2005) Homeodomain leucine zipper class I genes in Arabidopsis. Expression patterns and phylogenetic relationships. *Plant Physiology* **139**, 509–518.

He LQ, Tang RH, Jiang J, Xiong FQ, Huang ZP, *et al.* (2017) Rapid gene expression change in a novel synthesized allopolyploid population of cultivated peanut × *Arachis doigoi* cross by cDNA-SCoT and HFO-TAG technique. *Journal of Integrative Agriculture* **16**, 1093–1102.

Jain SM, Al-Khayri JM and Johnson DV (2011) *Date Palm Tissue Culture: A Pathway to Rural Development*. Date Palm Biotechnology. Springer Science and Business Media. https://doi.org/10.1007/978-94-007-1318-5_3.

Jia W, Yao Z, Zhao J, Guan Q and Gao L (2017) New perspectives of physiological and pathological functions of nucleolin (NCL). *Life Sciences* **186**, 1–10.

Khaled KA, El-Arabi NI, Sabry NM and El-Sherbiny S (2018) Sugarcane genotypes assessment under drought condition using amplified fragment length polymorphism. *Biotechnology* **17**, 120–127.

Krasensky J and Jonak C (2011) Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *Journal of Experimental Botany* **63**, 1593–1608. https://doi.org/10.1093/jxb/err460.

Lin Z, Li Y, Zhang Z, Liu X, Hsu C C, *et al.* (2020) A RAFSnRK2 kinase cascade mediates early osmotic stress signaling in higher plants. *Nature Communications* **11**, 1–10.

Luo C, He XH, Hu Y, Yu HX, Ou SJ, *et al.* (2014) Oligo-dT anchored cDNA–SCoT: a novel differential display method for analyzing differential gene expression in response to several stress treatments in mango (*Mangifera indica* L). *Gene* **548**, 182–189.

Maksymiec W (2007) Signaling responses in plants to heavy metal stress. *Acta Physiologiae Plantarum* **29**, 177–187.

Mizoi J, Kanazawa N, Kidokoro S, Takahashi F, Qin F, *et al.* (2019) Heat-induced inhibition of phosphorylation of the stress protective transcription factor DREB2A promotes thermotolerance of *Arabidopsis thaliana*. *Journal of Biological Chemistry* **294**, 902–917.

Mulpuri S, Muddanuru T and Francis G (2013) Start codon targeted (SCoT) polymorphism in toxic and non-toxic accessions of *Jatropha curcas* L. and development of a codominant SCAR marker. *Plant Science* **207**, 117–127.

Munier P (1973) Le palmier-dattier. Paris, Maisonneuve et Larose, 221 p.

Munns R and Tester M (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology* **59**, 651–681.

Parida AK and Das AB (2005) Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety* **60**, 324–349.

Rhouma-Chatti S, Choulak S, Moussa M, Chatti K and Chatti N (2020) Gene-targeted markers to assess genetic diversity and population structure within Tunisian *Phoenix dactylifera* L. cultivars. *Silvae Genetica* **69**, 29–35.

Ribone PA, Capella M, Arce AL and Chan RL (2016) What do we know about homeodomain–leucine zipper I transcription factors? Functional and biotechnological considerations. In *Plant Transcription Factors*, Academic Press. Chapter 22, pp. 343–356.

Sabri A, Bouaziz A, Hammani A, Kuper M, Douaik A, *et al.* (2017) Effet de l'irrigation déficitaire contrôlée sur la productivité et l'efficience d'utilisation de l'eau du palmier dattier cv Majhoul. *Revue Marocaine des Sciences Agronomiques et Vétérinaires* **5**, 23–31.

Sawant SV, Singh PK, Gupta SK, *et al.* (1999) Conserved nucleotide sequences in highly expressed genes in plants. *Journal of Genetics* **78**, 123–131.

Slama I, Abdelly C, Bouchereau A, Flowers T and Savouré A (2015) Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Annals of Botany* **115**, 433–447.

Tuteja R and Tuteja N (1998) Nucleolin: a multifunctional major nucleolar phosphoprotein. *Critical Reviews in Biochemistry and Molecular Biology* **33**, 407–436.

Waterworth WM, Kozak J, Provost CM, Bray CM, Angelis K J, *et al.* (2009) DNA ligase 1 deficient plants display severe growth defects and delayed repair of both DNA single and double strand breaks. *BMC Plant Biology* **9**, 1–12.

Wu JM, Li Y, Yang LT, Fang FX, Song HZ, *et al.* (2013) cDNA-SCoT: a novel rapid method for analysis of gene differential expression in sugarcane and other plants. *Australian Journal of Crop Science* **7**, 659–664.

Xiong K, Schumaker S and Jian-Kang Z (2002) Cell signaling during cold, drought, and salt stress liming. *The Plant Cell* **14**, 165–183.

Xiong FQ, Tang RH, Chen ZL, Pan LH and Zhuang WJ (2009) SCoT: a novel gene targeted marker technique based on the translation start codon. *Molecular Plant Breeding* **7**, 635–638.

Yaish MW and Kumar PP (2015) Salt tolerance research in date palm tree (*Phoenix dactylifera* L.), past, present, and future perspectives. *Frontiers in Plant Sciences* **6**, 348.

Yoshimura K, Mori T, Yokoyama K, Koike Y, Tanabe N, *et al.* (2011) Identification of alternative splicing events regulated by an arabidopsis serine/arginine-like protein, atSR45a, in response to high-light stress using a tiling array. *Plant Cell Physiology* **52**, 1786–1805.

Zohary D, Hopf M and Weiss E (2012) *Domestication of Plants in the Old World*, 3e édition. New York: Oxford University Press, p. 264.

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