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Gene Expression Profiling in Halwani and Baladi Grapevine (*Vitis vinifera* L.) Cultivars under Saline Conditions

Basel Saleh* **and Eyad Alshehada**

Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria (AECS), P.O. Box 6091, Damascus, Syria

***Corresponding author:** Saleh B, Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria (AECS), P.O. Box 6091, Damascus, Syria, Tel: 00963112132580, E-mail: bsaleh@aec.org.sy

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Abstract

Transcriptional patterns of one Na^+/H^+ antiporter (VvNHX1) gene, two AREB/ABF (VvAREB2 and VvABF1) genes, one DREB/CBF (VvCBF4) gene and one Osmotin-like protein (VvOSM1) gene were evaluated in Halawani and Baladi grapevine (*Vitis vinifera* L.) cultivars leaves after 1, 3 and 5 days of exposure to 2 dS/m seawater (SW) using Real-Time PCR (RT-qPCR) tool. Varietal difference in the VvNHX1, VvAREB2, VvABF1, VvCBF4 and VvOSM1 transcripts amounts has been observed between saline conditions and non-saline ones. In this regards, VvABF1 and VvAREB2 transcripts showed up-regulation in the studied cultivars and similarly for VvNHX1 in Halawani cv, when exposure time increased from 1 to 5 days. However, VvOSM1 transcripts showed inverse trends in Baladi cv. whereas, VvCBF4 showed up-regulation after 1 and 3 days of exposure time in the studied cultivar. Five days after exposure it continuously increased in Halawani cv. and decreased in Baladi cv. Thereby, RT-qPCR analysis successfully highlighted varietal difference in the above genes transcript accumulation in response of grapevine to saline conditions.

Keywords: Grapevine (*Vitis vinifera* L.); Salt stress; Real-Time PCR (RT-qPCR) technique; Transcript analysis

Introduction

Grapevine (*Vitis vinifera* L.) is considered to be one of the major fruit crops through the world due to its cultivated hectares, religious and economic value and multiuse (fresh fruit, dried fruit, juice and wine production) [1,2]. It has been demonstrated that its cultivation is ancient for more than 5000 years in Syria according to unique historical and geographical emplacement [3]. In Syria, Halawani followed by Baladi grapevine cultivars were considered among the 100 grapevine cultivars grown in Syria, are the most cultivated ones [4] with production is approximately estimated to be 30 and 20%, respectively from the total Syrian grapevine production [5]. Salinity is a serious constraint continuously increased worldwide. Salinity as an abiotic stress adversely affects biological processes, metabolism inhibition, plant growth and development reduction, RNA, DNA and protein damages and gene expression alteration [6-8]. Gene expression changes (upregulation or downregulation) can lead to enhanced plants salinity tolerance [7-9]. Salinity tolerance is known as a complex trait controlled by many genes. These genes e.g., NHX1, DREB, ABRE, CDPK and osmotin or osmotin-like protein such as OSM1 belong to different families. These proteins displayed a potential role in plant growth and development and mediate gene expression profiling after abiotic stress exposure [8,10-13]. NHX1 is a gene that encodes vacuolar Na^+/H^+ antiporter. It has been demonstrated that in plants, Na^+/H^+ antiporter facilitates removing of Na by transporting it from the cytoplasm to the vacuole through electrochemical H+ gradient [14]. Thereby, it plays an important role in the alleviation of unfavorable effects of salt towards plants. In this respect, it was cloned and characterized in many plant crops e.g., in grapevine (VvNHX1) [6,10]; *Arabidopsis thaliana* (AtNHX) [14]; sugar beet (*Beta vulgaris* L.) (BvNHX1) [15] and Citrus (CNHX1) [16].

As for AbsCisic Acid Response Element (ABRE)-binding protein (AREB)/ABRE binding factors (ABFs), among basic domain leucine zipper (bZIP) transcription factors (TFs), are activated in the presence of abscisic acid (ABA) and regulate the expression of genes by binding to the ABRE in their promoter regions [8]. The previous research clearly reported the important role of the mentioned genes in salinity adaptation "Various TFs regulate the expression of ABA-responsive genes through ABRE, which contains the core sequence PyACGTGGC that regulates dehydration- and high salinity responsive gene expression in plants in the ABA-dependent pathway". It has been demonstrated that among AREB/ABFs protein, AREB1/ABF2, AREB2/ABF4, and ABF3 were inducible by dehydration, high salinity, or ABA treatment in vegetative tissues [17,18].

Whereas, the dehydration responsive element-binding (DREB) DREB/CBFs genes were known as low temperature responsive genes. However, their expression profiles altered in some plants according to various stresses conditions [8], where, ABA activate some DREB/CBF genes [8,9]. Whereas, Zhao et al. [11] characterized 38 VvDREB members from the whole grapevine genome. More recently, Khan et al. [12] successfully described in detail the important role of transcription factors (TFs) for regulating gene expression and thereby, enhancing plants abiotic stress tolerance.

To our knowledge, there is a lack of information on the examination of the above-mentioned genes in grapevine under saline conditions. Thereby, the expression profiling of five genes (VvNHX1, VvAREB2, VvABF1, VvCBF4 and VvOSM1) was undertaken in leaves of two Halawani and Baladi grapevine cultivars under 2 dS/m seawater (SW) after 1, 3 and 5 days of exposure using Real-Time PCR (RT-qPCR) technique.

Materials and Methods

Preparation of plant material

Baladi and Halawani grapevine cultivars were obtained from the General Commission for Scientific Agricultural Research of Syria (GCSAR) providing source of cultivars multiplication. Preparation of plant materials and experimental conditions were as recently described by Saleh and Alshehada [13]. Plants were watered with tap water twice per week for two months before salt stress treatment. Some plants were continuously watered with tap water as a control. Whereas, other ones were watered with 2 dS/m sea water (SW). The experiment (three replicates per treatment) was carried out in the greenhouse for 5 days. Leaf samples (for control and stressed plants) were harvested 1, 3 and 5 days after salt treatment and were kept frozen in liquid nitrogen.

Isolation of RNA and cDNA synthesis

RNA extraction and cDNA synthesis were performed as recently reported by Saleh and Alshehada [13]. Synthesized cDNA was kept at -20 °C until use.

Quantitative real-time PCR (qPCR) test

Expression profile of VvNHX1, VvAREB2, VvABF1, VvCBF4 and VvOSM1 genes was evaluated in Halwani and Baladdi grapevine cultivars after different times of exposure to 2 dS/m SW. Quantitative RT-qPCR assay was carried out by using the VvNHX1 gene 5'-GGATCACCCACTGAACCAAA-3' (VvNHX1F) and (5'-TTCACACAGAAAGCCGATTG-3') (VvNHX1R) [6], VvABF1 gene 5'-TGATAAACACATGGCTGACC-3' (VvABF1F) and 5'-TCTTCCAAAGTCATCTCCCC-3' (VvABF1R) [8], VvAREB2 gene 5'-TAACCACATTAGCAACTCCC-3' (VvAREB2F) and 5'-CATTATGAACGCTGTCCTGC-3' (VvAREB2R) [8], VvCBF4 gene 5'-ACCCTCACCCGCTCGTATG-3' (VvCBF4F) and 5'-CCGCGTCTCCCGAAACTT-3' (VvCBF4R) [8] and the VvOSM1 gene 5'-AACTCAACAATGGGCCTCTG-3' (VvOSM1F) and 5'-TGCAACCACCGGTAGTCTTT-3' VvOSM1R [19]. Whereas, the VvEF1α gene was chosen as a housekeeping gene and amplified with the forward primer F (5′-CGGGCAAGAGATACCTCAAT-3′) and the reverse primer R (5′- AGAGCCTCTCCCTCAAAAGG -3′) [8]. Real-Time qPCR assay has been performed using Rotor-Gene Q (ABI Applied Biosystem) with 96-well rotor, and the FastStart SYBR Green Master kit (Thermo), with the recommended thermal profile (40 cycles). Real-Time qPCR test was carried out as recently described by Saleh and Alshehada [13].

Semi-Quantitative real-time PCR (PCR) data analysis

RT-qPCR products were electrophoresed as recently described by Saleh and Alshehada [13]. Gel documentation 2000 USA was used to estimate band intensity.

Results and Discussion

Expression patterns of five genes (VvNHX1, VvAREB2, VvABF1, VvCBF4 and VvOSM1) have been investigated after various periods of time (1, 3 and 5 days) in leaves of Halawani and Baladi grapevine cultivars under 2 dS/m seawater (SW) based on RT-qPCR technique. Data revealed that the five tested genes followed different manners according to the examined cultivar, gene and exposure time (Figure 1).

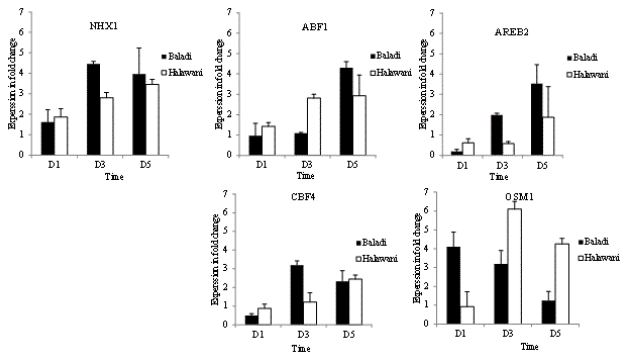

Figure 1: Relative expression profiles of NHX1, ABF1, AREB2, CBF4 and OSM1 genes in Baladi and Halawani grapevine cultivars leaves after 1D, 3D and 5D (days) of exposure to 2 dS/m seawater (SW) using RT-qPCR test. Error bars are representative of the standard error of the mean of the three replicates.

Each gene behavior is separately discussed. VvOSM1, showed down-regulation from 4.1 to 1.25-fold when exposure time increased from 1 to 5 day in Baladi cv. Whereas, its expression showed up-regulation from 0.92 to 6.1-fold when exposure time increased from 1 to 3 day followed by down-regulation to 4.25-fold after 5 day of exposure in Halawani cv. As for VvABF1, showed similar trends of up-regulation in Baladi cv. (from 0.96 to 4.3-fold) and in Halawani cv. (from 1.4 to 2.9-fold) when exposure time increased from 1 to 5 day. VvNHX1, showed up-regulation from 1.6 to 4.5-fold when exposure time increased from 1 to 3 day with decrease to 4-fold in Baladi cv, after 5-day exposure. Whereas, it showed up-regulation in its expression from 1.9 to 3.5-fold in Halawani cv when exposure time increased from 1 to 5 day. As for VvAREB2, it showed up-regulation from 0.2 to 3.5-fold and from 0.6 to 1.9-fold in Baladi cv and Halawani cv., respectively, when exposure time increased from 1 to 5 day. VvCBF4, showed up-regulation from 0.5 to 3.2-fold after 1 and 3 days of exposure time with down-regulation to 2.3-fold after 5 day of exposure in Baladi cv. Whereas, it showed up-regulation from 0.9 to 2.4-fold when exposure time increased from 1 to 5 day in Halawani cv. Data revealed varietal variation in transcripts levels. In this regard, for VvABF1 and VvAREB2, their expression pattern increased progressively with prolongation exposure of time from 1 to 5 days in the two cultivars. Whereas, for VvNHX1, and VvCBF4, showed also similar trends, where their expression increased as exposure time increased from 1 to 3 days, and then their expression is down regulated in the both cultivars. However, for VvOSM1 it followed similar trends observed with VvABF1 and VvAREB2 in the case of Baladi cv, whereas, it followed similar trends observed with VvNHX1 and VvCBF4 in the case of Halawani cv. These observed changes in the expression level in both studied cultivars might reflect in changes of band gene intensity towards 2 dS/m seawater (SW) after 1, 3 and 5 days of exposure using semiquantitative RT-qPCR test (Figure 2). Hanana et al. [10] reported that VvNHX1 expression of cv. Cabernet Sauvignon grapevine, partially complements the salt- and hygromycin-sensitive phenotypes of an ena1-4 nhx1 yeast strain.

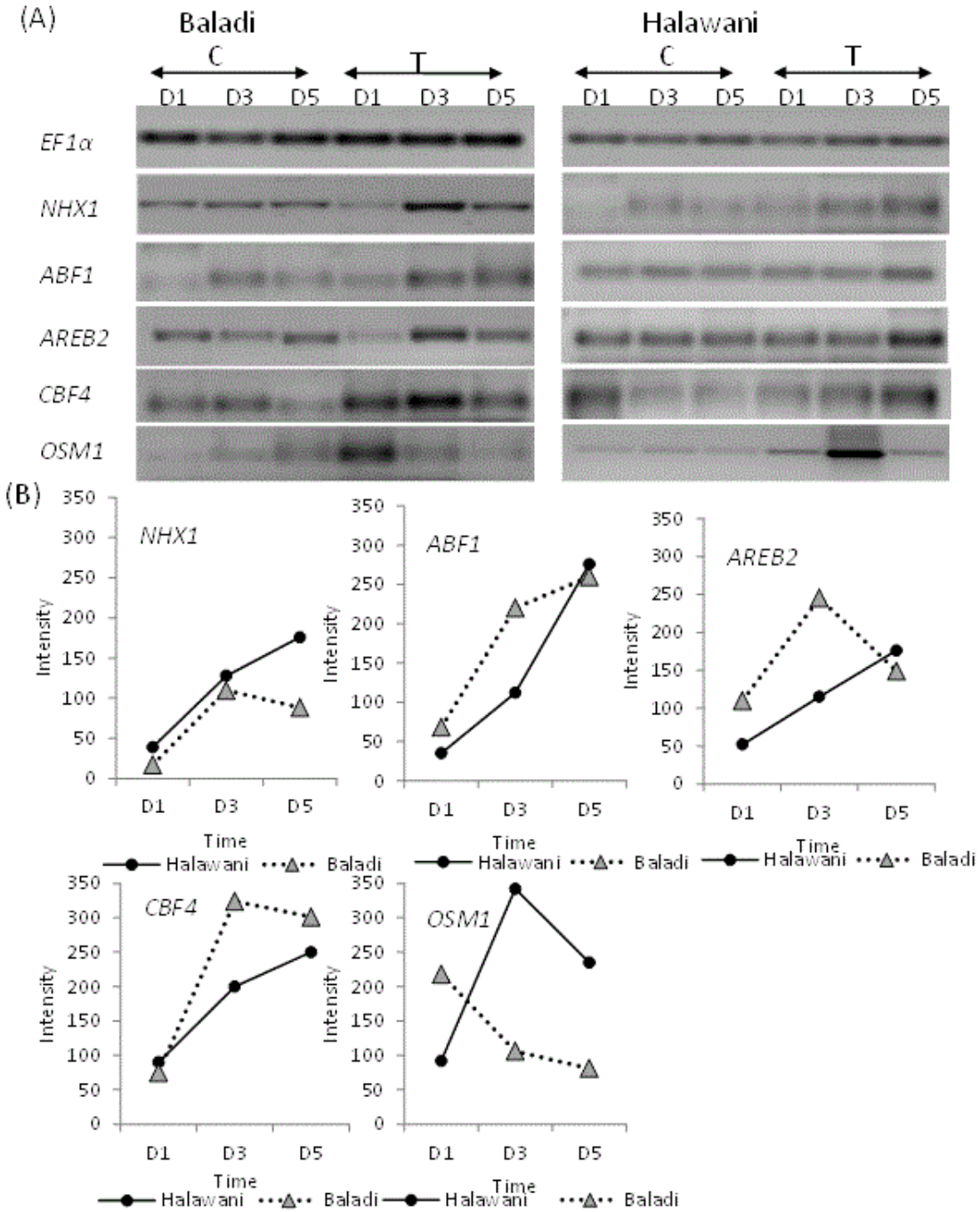

Figure 2: Expression patterns of NHX1, ABF1, AREB2, CBF4 and OSM1 genes in Baladi and Halawan grapevine cultivars leaves after 1D, 3D and 5D (days) of exposure to 2 dS/m seawater (SW) using semi-quantitative RT-PCR (A). Changes in band genes intensity after different time intervals (B). C: Control; D1: 1 day, D3: 3 days and 5D: 5 days. T: Treated plants.

Whereas, Upadhyay et al. [6] reported VvNHX1 gene expression in two 110R and 1613C grapevine rootstocks subjected to 2 and 4 dS/m for 7 and 21 days. The previous investigation revealed that the VvNHX1 gene expression increased about 1.3 and 4.5-fold after 7 and 21 days exposure, respectively in 110R indicating up-regulation of this gene. Whereas, decline in its expression has been observed for 1613C regardless time interval indicating down-regulation of this gene. Xiao et al. [9] reported the up-regulation of CBF4 gene expression under low temperature, drought, and salinity in *V. vinifera* and *V. riparia.* They reported that CBF4 was expressed in Vitis sp. under cold stress that provoked an osmotic stress. The expression has been also observed in *Medicago truncatula* under cold, drought and high salt stresses, and

ABA treatment [7]. The later study mentioned that the MtCBF4 expression in *M. truncatula* based on microarray assay, has been induced by salt, drought, cold, and abscisic acid stresses. Moreover, the previous study revealed that the MtCBF4 overexpression improved drought and salt stress tolerance in transgenic Arabidopsis plants. Whereas, Zhao et al. [11] successfully clarified 38 VvDREB members from the whole grapevine genome. The previous study revealed that these genes were distributed in 15 of 19 chromosomes in grapevine. Moreover, microarray test employed to investigate transcript patterns in grapevine indicated that VvDREB23 overexpression showed that 229 genes were down-regulated and 248 were up-regulated, with fold-changes of >1.5 than the empty vector control. Zandkarimi et al. [8] reported AREB/ABF genes in five grape varieties [Qalati (Q), Kaj Angoor (KA), Sabz Angoor (SA), Siahe Zarghan (SZ), Bidane Safid (BS)] subjected to salt stress (10 and 20 dS/m NaCl for 9 and 19 days period) and drought stress (-0.3 MPa, -0.7 MPa, -1.0 MPa and -1.5 MPa). The previous investigation reveale that the AREB2 was up-regulated with increasing salt for all examined varieties. Whereas, it showed down-regulation under 10 dS/m NaCl for Q variety. As for ABF1, it was up-regulated at 10 dS/m NaCl for all examined varieties. Whereas, different regulation patterns have been recorded; e.g., at 20 dS/m NaCl, slight down-regulation (0.9-fold) was recorded for SA. Indeed, this study showed down-regulation about 0.3-fold for BS. While, its expression inversely increased for SZ; moreover, it was up-regulated at 20 dS/m NaCl for KA and Q. Moreover, the later study mentioned the DREB/CBF genes expression level in the mentioned five grape varieties subjected to salt and drought stresses. The previous investigation revealed different transcript patterns in the examined five grape varieties according to the applied stress type. In this regard, more expression level has been recorded for CBF1 and CBF3 under salinity stress. However, CBF2 responded under salinity and drought stresses, whereas, CBF4 was up-regulated under salinity and drought stresses regardless of examined tissues (leaf and root). Its expression increased as NaCl applied concentration increased except for SZ, where it showed a decline expression at 20 dS/m NaCl compared to 10 dS/m NaCl. Moreover, Yoshida et al. [18] reported that AREB2 was up-regulated by water stress and ABA. Other investigations also revealed that AREB/ABF-overexpressing in plants played an important role in improving abiotic stresses tolerance [8,17,20]. Whereas, Choi et al. [21] and Boneh et al. [22] reported the induction of AREB1/ABF2 and ABF3 expression genes under high saline conditions. Indeed, they reported the induction of AREB2/ABF4 expression gene under high salt, cold, and drought in Arabidopsis plants. These observations indicate that AREB1/ABF2 and ABF3 genes were involved in high salt signals transduction, whereas, AREB2/ABF4 is involved in multiple stress responses [21]. Whereas, Agaolu et al. [19] reported the VvOSM1 gene expression patterns in 10 cultivars and 7 rootstocks grapevine subjected to different NaCl (0.2, 0.5, 0.7 and 1.3 dS/m) concentrations after different times periods (1, 3.5 and 8 days) through Northern blot test. The previous study revealed that VvOSM1 gene expression level was higher in cultivars than in rootstocks. Indeed, genotyping variation in its expression level has been observed among the tested 10 cultivars. More recently, Saleh and Alshehadah [13] reported that VvOSM1 transcript level progressively increased with continuously manner as salt concentration and exposure time period increased. In this regard, in Baladi cv. its expression level was up-regulated by 4, 4.1 and 10.5-fold under 1, 2 and 3 dS/m SW, respectively; whereas, in Halwani cv., its expression was up-regulated by 1.2, 5.8 and 7.2-fold under 1, 2 and 3 dS/m SW, respectively after 3 days of exposure. Indeed, its expression after 5 days of SW exposure, was continuously increased in Halawani cv. by 2.5, 4.4 and 8.3-fold under 1, 2 and 3 dS/m SW, respectively. Indicating that its expression became maximum at the end of experiment in Halwani cv. Whereas, in Balabi cv, its expression increased after 1 day and notably after 3 days exposure. While, after 5 days exposure its expression decreased indicating that this gene was activated after 3 days and inhibited with prolonged period over than 3 days.

Conclusion

In the current study, VvNHX1, VvAREB2, VvABF1, VvCBF4 and VvOSM1 transcriptional profile was evaluated in two Halawani and Baladi grapevine cultivars after 1, 3 and 5 days of exposure to 2 dS/m SW. Briefly, ABF1 and AREB2 transcripts showed up-regulation for the both examined cultivars and similarly for NHX1 in Halawani cv, when exposure time increased from 1 to 5 day. However, OSM1 transcripts showed inverse trends in Baladi cv. To confirm the results obtained herein, it is worth noting that abscisic acid (ABA), salicylic acid (SA), methyl jasmonate (MJ), ethylene, proline, catalase (CAT), superoxide dismutase (SOD), ascorbate (ASC) and Glycine betaine (GB) measurement is needed.

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Conflict of Interest

The authors declare that the study involved no conflict of interest from any party.

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