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Research Article

Abscisic Acid (ABA) and Salicylic Acid (SA) Content in Relation to Transcriptional Patterns in Grapevine (*Vitis vinifera* L.) under Salt Stress

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ABSTRACT

Correlation between two phytohormone Abscisic acid (ABA) and salicylic acid (SA) accumulation and transcript pattern of VvNHX1, VvABF1, VvAREB2, VvCBF4 and VvOSM1 genes involved in grapevine (*Vitis vinifera* L.) adaptation against 2 dS/m seawater treatment for 1,3 and 5 days exposure has been assessed in Baladi and Halawani cultivars and B41 rootstock grapevine. Data revealed that VvNHX1, VvABF1, VvAREB2 and VvCBF4 transcript patterns were closely related with activation of ABA level in Halawani cv. Whereas, VvABF1 and VvAREB2 transcript patterns were closely related with activation of ABA level in Baladi cv. While, VvNHX1, VvABF1 and VvCBF4 transcript patterns were closely related with activation of ABA level in B41 rootstock. Indeed, VvOSM1 transcript pattern was closely related with ABA level in B41 rootstock. As for SA, VvNHX1 and VvCBF4 transcript patterns were closely related with SA level in Baladi cv. Whereas, VvAREB2 and VvABF1 transcript pattern was closely related with activation of SA level in B41 rootstock. While, VvOSM1 transcript pattern was closely related with activation of SA level in Halawani cv. However, its transcript pattern was inversely related with SA level in Baladi cv. Thereby, the current investigation suggests that transcript pattern of some genes were closely related with activation of ABA or SA or the both together; indicating that these genes could implicated in phytohormones pathway mediates salt tolerance in grapevines.

Keywords: Grapevine; Salt stress; Transcript pattern; Abscisic acid (ABA); Salicylic acid (SA)

INTRODUCTION

Valuable importance and multiuse of grapevine (*Vitis vinifera* L.) encourage its cultivation worldwide. In Syria, it was cultivated since 5000 years, and that Halawani and Baladi grapevine cultivars were the most important and cultivated once among the 100 grown grapevine cultivars [1,2].

Salinity as an abiotic stress caused morphological, physiological, biochemical and genetic alterations of which genes expression profile changes are among them. Many genes such AREB1, AREB2 and ABF3 could induce after adverse abiotic stresses like salinity and drought and that ABA acts as a gene regulator suggesting that this element could involve in stress signaling [3]. Overall, plants developed adverse mechanisms at different levels (morphological, physiological, biochemical, molecular..ect.) in response to biotic and abiotic stresses including wide range of signal transduction pathways initiation. Salicylic acid (SA) among them proved to play a significant role in enhancing plants salinity tolerance due to its role in regulating genes expression pathways by extensive signaling cross-talk with other growth substances [3-6].

It has been documented that that DREB/CBF genes are inducible by adverse stresses, and that ABA acts as an endogenous messenger in plant and in activating some DREB/CBF genes [7,8]. Moreover, AREB/ABFs are involved in ABA and stress signaling in *Arabidopsis*, rice and grapevine [9-11].

Yoshida et al. [9] reported that AREB/ABFs function predominantly in stress-responsive gene expression downstream of SRK2D/E/I. Of which, AREB1, AREB2, ABF3 and ABF1 genes were mainly transcription factors of SRK2D/E/I in ABA signaling as responsive osmotic stresses during vegetative stage.

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ABA is a universal plant hormone proved to regulate plant growth and development processes and different genes expression under adverse abiotic stresses [12-15]. As for salicylic acid (SA) is a phenolic phytohormone involved in plants adaptation against different environmental stresses like salinity and act as signaling molecule in abiotic stress tolerance enhancement. Recently, its capital role in salinity tolerance enhancement has been reviewed in wide range of plant species e.g in wheat (*Triticum aestivum*), tomato (*Lycopersicon esculuntum* Mill.), maize (*Zea mays* L.), *Medicago sataiva*, sunflower (*Helianthus annuus* L.), Torreya grandis, Arabidopsis thaliana, Chickpea (*Cicer arietinum* L.), white bean (*Phaseulus vulgaris*), strawberry (*Fragaria ananassa*), barley (*Hordeum volgaris*) and mungbean (*Vigna radiata* L.), *Medicagosativa* [16,17] and in grape (*Vitis vinifera* L.)[3]. Moreover, Roustakhiz and Saboki [18] reported its important role on yield and its components under salt stress in grapevine (*Vitis vinifera*). More recently, Husen et al [19]. Reported its role for alleviate adverse negative effect induced by salt stress in Ethiopian mustard (*Brassica carinata* A. Br.).

Phytohormones such as abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) have been implicated in responses to osmotic stresses. For this reason the majority of published articles mainly focused on their application effects to alleviate unfavorable deleterious effects of osmotic stresses like salinity and drought. However, little attention has been carried out regarding their accumulation level in plants after exposure to osmotic stresses. Thereby, the current investigation emphasized their accumulation level correlated with transcriptional patterns in grapevine after seawater treatment. In this regards, to earlier, Downton and Loveys [20] reported ABA level in *V. vinifera* L. leaves after exposure to NaCl. Whereas, Jia et al. [21]reported ABA accumulation in maize (*Zea mays* cv. Zhongyou 1) under NaCl treatment. Moreover, Ismaila et al. [22] reported the correlation between ABA and JA concentration and osmotic stress (120 mM mannitol) in two *Vitis* cell lines differ in their salt tolerance capacity.

MATERIALS AND METHODS

Transcriptional pattern assay

Preparation of plant material including Baladi and Halawani cultivars and B41 rootstock grapevine, salt stress application, RNA extraction and cDNA synthesis, quantitative real-time PCR (qPCR) and semi-Quantitative tests were performed as recently described by Saleh and Alshehada [1,2].

ABA and SA determination

Leaf samples (for control and stressed plants) were harvested 1, 3 and 5 days after SW treatment. Approximately 200 mg of freshly ground leaves following the method of Trapp et al.[23], with minor modifications. Samples were kept frozen at -42°Covernight. One mL of ethyl acetate, dichloromethane, isopropanol, MeOH:H_2O was added to each sample. Samples were centrifuged for 5 min at 16000 g/4°C after shaken for 30 min. The resulting supernatant was transferred to a new tube and dried in a speed vacuum. One hundred µL of MeOH was added to each sample and centrifuged for10 min at 16000 g/4°C. High-performance liquid chromatography coupled mass spectrophotometer (HPLCMS/MS) system (Agilent Technologies, Boblingen, Germany).

Was used to ABA and SA quantification. Alteration in ABA and SA content was compared to the control for each time point. Three replicates for each sample were performed for each time point. Data were expressed as mean ± standard deviation and t-test methods.

RESULTS AND DISCUSSION

Visible injury symptoms induced by SW treatment were recorded 1, 3 and 5 days after SW treatment (Figure 1). Leaf necrosis was observed into the three studied accessions combined with leaf burn into B41 rootstock only.

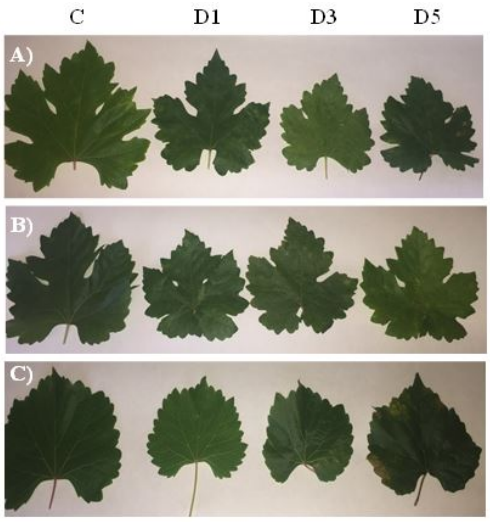

Figure 1: Morphological change induced by 2 dS/m SW in Baladi (A) and Halwani (B) cultivars and B41 (C) rootstock grapevine leaves after 1, 3 and 5 days of SW exposure. C: Control; D1:1 day, D3:3 days and 5D:5 days.

Alteration in total ABA content was presented in Figure 2.

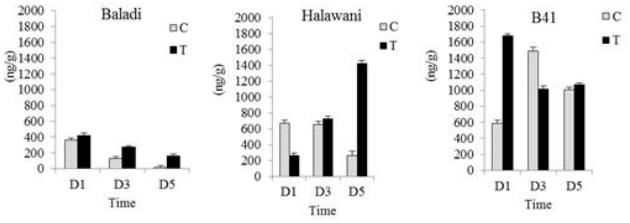

Figure 2: Total abscisic acid (ABA)content inin Baladi and Halawani cultivars and B41 rootstock grapevine leaves after 1D, 3D and 5D (days) of exposure to 2 dS/m seawater (SW). C:Control; D1:1 day, D3:3 days and 5D:5 days. T:Treated plants. Error bars represent the standard error of the means (n=3).

Whereas, total SA change content was presented in Figure 3.

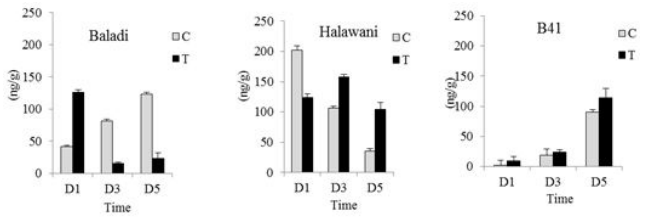

Figure 3: Total salycilic acid (SA) content inin Baladi and Halawani cultivars and B41 rootstock grapevine leaves after 1D, 3D and 5D (days) of exposure to 2 dS/m seawater (SW). C: Control; D1:1 day, D3:3 days and 5D:5 days. T:Treated plants. Error bars represent the standard error of the means (n=3).

Data revealed that VvNHX1, VvABF1, VvAREB2 and VvCBF4 transcript patterns were closely related with activation of ABA level in Halawani cv. Whereas, VvABF1 and VvAREB2 transcript patterns were closely related with activation of ABA level in Baladi cv. While, VvNHX1, VvABF1 and VvCBF4 transcript patterns were closely related with activation of ABA level in B41 rootstock (Figure 4A). Indeed, VvOSM1 transcript pattern was closely related with ABA level in B41 rootstock (Figure 4A). As for SA, VvNHX1 and VvCBF4 transcript patterns were closely related with SA level in Baladi cv. Whereas, VvAREB2 and VvABF1 transcript pattern was closely related with activation of SA level in B41 rootstock (Figure 4B). While, VvOSM1 transcript pattern was closely related with activation of SA level in Halawani cv. However its transcript pattern was inversely related with SA level in Baladi cv. (Figure 4B).

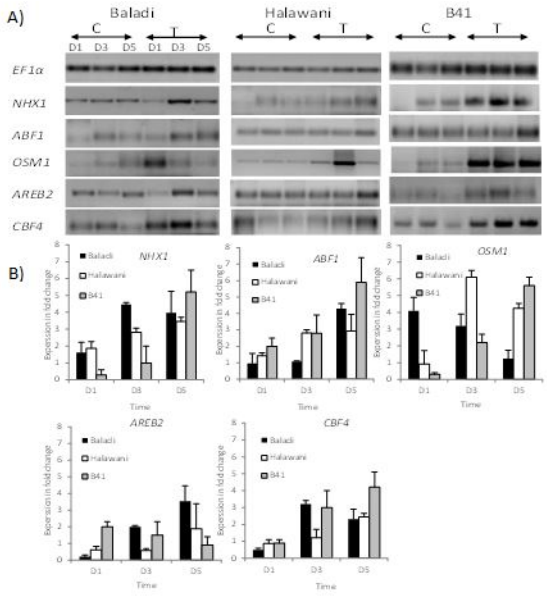

Figure 4: Semi-quantitative RT-PCR (A) and quantitative RT-PCR (B) expression profile change of NHX1, ABF1, AREB2, CBF4 and OSM1 genes in Baladi and Halawani cultivars and B41 rootstock grapevine leaves after 1D, 3D and 5D (days) of exposure to 2 dS/m seawater (SW). C:Control; D1:1 day, D3:3 days and 5D:5 days T:Treated plants.

Effect of 2 dS/m seawater treatment for 1,3 and 5 days exposure has been assessed in Baladi and Halawani cultivars and B41 rootstock grapevine, by monitoring morphological effect. Data showed leaf necrosis was observed into the three studied accessions combined with leaf burn into B41 rootstock only. Baneh et al. [24,25] reported a visible injury symptoms (leaf burn and necrosis) in 9 wild *Vitis vinifera* genotypes (*Vitis vinifera* ssp. *sylvestris*) followed NaCl treatment; and that these damages increased in all studied genotypes with salt treatment increased from 50 mM to 150 mM NaCl. Similarly, sinclair and Hoffmann [26] reported leaf burn and senescence in *Vitis vinifera* L. cv. Shiraz (rootstock 3306) rootstock under saline conditions (0.7-4.1 dS/m).

Plants developed various mechanisms at different levels to enhancing their capacity defense systems e.g. through antioxidant accumulation like abscisic acid (ABA) and salicylic acid (SA). To earlier, Giraudat et al. [27] reported that VvAREB1 and VvABF2 genes are among the most important transcription factors involved in plants ABA signaling pathways. Whereas, Yoshida et al. [9] reported that ABA a plant hormone displayed a critical role in stress-responsive genes expression mainly through three bZIP transcription factors, AREB1/ABF2, AREB2/ABF4 and ABF3, that are activated by SNF1-related kinase 2s (SnRK2s) after plant exposure to osmotic stresses e.g. drought and salinity. Moreover, Ismail et al. [22] reported that the ABA-activated SnRK2s phosphorylate downstream transcription factors e.g. the ABA responsive element-bindingprotein (AREB) and ABA responsive element-binding factor (ABF) and bZIP-type leading to activating ABA-responsive genes and ABA-related responses. They reported that ABA level increased in both salt-sensitive *Vitis riparia* and *Vitis rupestris* salt-sensitive grapevine cell lines after 1, 3 and 6 h exposure to 120 mM mannitol. This increase was drastically to 4-folds after 3 to 6 h exposure in *V. rupestris*; whereas, it was less pronounced in *V. riparia*.

Jia et al. [21] reported that ABA accumulation in maize (*Zea mays* cv. *Zhongyou* 1) root tissues (up to 10-fold) was comparable with that reported in leaf once (about 1-fold) under NaCl treatment for 15 min. While, Shi and Zhu [28] investigated Na^+/H^+ accumulation under osmotic stress in *Arabidopsis* plants. They reported that osmotic stress activates the ABA synthesis leading to up-regulate the AtNHX1 transcription.

Previously, Downton and Loveys [20] reported increase in ABA level has been recorded in *V. vinifera* L. leaves after 6 h of exposure to 50 and 100 mM NaCl. Indeed, Cramer et al. [29] reported increase ABA amount under water and salinity stresses in in grapevine leaves. Inversely, Ohori and Fujiyama [30] reported rapid chute in ABA level when NaCl concentration increased from 0.005 to 0.5 mol/m NaCl in *Salicornia bigelovii* halophytic shoot.

It has been documented that DREB/CBF genes are also inducible by adverse stresses, and that ABA acts as an endogenous messenger in plant and in activating some DREB/CBF genes [7,8]. In this regards, Boneh et al [11] stated that tissue-specific expression of VvABFs was recorded after various abiotic stresses. They reported that VvABF1 was highly

induced in roots, whereas, VvABF2 was up-regulated in the leaves under drought, salt and ABA stresses.

CONCLUSION

Phytohormone ABA and SA implement in transcript pattern of VvNHX1, VvABF1, VvAREB2, VvCBF4 and VvOSM1 genes involved in in Baladi and Halawani cultivars and B41 rootstock grapevinefollowed 2 dS/m seawater treatment for 1,3 and 5 days exposure has been investigated. Overall, among the studied gene transcript patterns, some of them closely related with activation of ABA or SA or the both together; referring that these genes could implicatedin phytohormones pathway mediates salt tolerance in grapevines. However, those that which not related with activation the mentioned phytohormones did not meaning that they have not any role in grapevine salt tolerance. This could explain by the fact that salt tolerance is a complex traits in which many genes expression are requested. So, the gene that does not activate the mentioned phytohormones, it may activate other genesthat did not investigate in the current study.

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CONFLICT OF INTEREST

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

REFERENCES

1. Saleh B, Alshehada E. Transcriptional analysis of VvOSM1 gene in grapevine (*Vitis vinifera* L.) under salt stress. J Plant Biochem Physiol. 2018;6(2):217-220.
2. Saleh B, Alshehada E. Gene expression profiling in Halwani and Baladi grapevine (*Vitis vinifera* L.) cultivars under saline conditions. J Plant Biochem Physiol. 2018;6(3):222-226.
3. Zandkarimi H, Ebadi A, Salami SA, Alizade H, Baisakh N. Analyzing the expression profile of AREB/ABF and DREB/CBF genes under drought and salinity stresses in grape (*Vitis vinifera* L.). PLoS ONE. 2015;10(7):0134288.
4. Asensi-Fabado M, Munné-Bosch S. The aba3-1 mutant of *Arabidopsis thaliana* withstands moderate doses of salt stress by modulating leaf growth and salicylic acid levels. J Plant Growth Regul. 2011;30:456-466.
5. Li G, Peng X, Wei L, Kang G. Salicylic acid increases the contents of glutathione and ascorbate and temporally regulates the related gene expression in salt-stressed wheat seedlings. Gene. 2013;529(2):321-325.
6. Khan MIR, Fatma M, Per TS, Anjum NA, Khan NA. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front Plant Sci. 2015;6:462.
7. Knight H, Zarka DG, Okamoto H, Thomashow MF, Knight R. Abscisic acid induces CBF gene transcription and subsequent induction of cold-regulated genes via the CRT promoter element. Plant Physiol. 2004;135:1710-1717.
8. Xiao H, Tattersall E, Siddiqua M, Cramer GR, Nassuth A. CBF4 is a unique member of the CBF transcription factor family of Vitis vinifera and Vitis riparia. Plant Cell Environ. 2008;31:1-10.
9. Yoshida T, Fujita Y, Maruyama K, Mogami J, Todaka D, Shinozaki K, et al. Four *Arabidopsis* AREB/ABF transcription factors function predominantly in gene expression downstream of SnRK2 kinases in abscisic acid signaling in response to osmotic stress. Plant Cell Environ. 2014;38:35-49
10. Hossain MA, Cho JI, Han M, Ahn CH, Jeon JS, An G, et al. The ABRE-binding bZIP transcription factor OsABF2 is a positive regulator of abiotic stress and ABA signaling in rice. J Plant Physiol. 2010;167:1512-1520.
11. Boneh U, Biton I, Schwartz A, Ben-Ari G. Characterization of the ABA signal transduction pathway in *Vitis vinifera*. Plant Sci. 2012;187:89-96.
12. Jamalian S, Gholami M, Esna-Ashari M. Abscisic acid-mediated leaf phenolic compounds, plant growth and yield is strawberry under different salt stress regimes. Theor Exper Plant Physiol. 2013;25(4):291-299.
13. Mohammadkhani N, Heidari R, Abbaspour N, Rahmani F. Effects of salinity on plant hormones genes in grape. Iran J Sci Technol Trans Sci. 2016;42:401-410.
14. Rattanakon S, Ghan R, Gambetta GA, Deluc LG, Schlauch KA, Cramer GR. Abscisic acid transcriptomic signaling varies with grapevine organ. BMC Plant Biol. 2016;16:72-85.
15. Ren C-G, Kong C-C, Xie Z-H. Role of abscisic acid in strigolactoneinduced salt stress tolerance in arbuscular mycorrhizal *Sesbania cannabina* seedlings. BMC Plant Biol. 2018;18:74-83.
16. Rajeshwari V, Bhuvaneshwari V. Salicylic acid induced salt stress tolerance in plants. Int J Plant Biol Res. 2017;5:1067-1072.
17. Palma F, López-Gómez M, Tejera NA, Lluch C. Salicylic acidimprovesthesalinitytoleranceof *Medicagosativa* in symbiosiswith *Sinorhizo biummeliloti* by preventing nitrogen fixation inhibition. Plant Sci. 2013;208:75-82.
18. Roustakhiz J, Saboki E. Effect of salicylic acid on yield and yield component of grapevine (Vitis vinifera) under salinity stress condition. Int J Farm Food Allied sci. 2017;6:39-42.
19. Husen A, Iqbal M, Sohrab SS, Ansari MKA. Salicylic acid alleviates salinity-caused damage to foliar functions, plant growth and antioxidant system in Ethiopian mustard (*Brassica carinata* A. Br.). Agri Food Sec. 2018;7:44-58.
20. Downton WJS, Loveys BR. Abscisic acid content and osmotic relations of salt-stressed grapevine leaves. Fun Plant Biol. 1981;8(5):443-452.
21. Jia W, Wang Y, Zhang S, Zhang J. Salt-stress-induced ABA accumulation is more sensitively triggered in roots than in shoots. J Exper Bot. 2002;53:2201-2206.
22. Ismaila A, Seo M, Takebayashi Y, Kamiya Y, Nick P. A balanced JA/ABA status may correlate with adaptation to osmotic stress in *Vitis* cells. J Plant Physiol. 2015;185:57-64.
23. Trapp MA, De Souza GD, Filho ER, Boland W, Mithofer A. Validated method for phytohormone quantification in plants. Frontiers in Plant Science. 2014;5:1-11.
24. Baneh DH, Hassani A, Shaieste FG. Effects of salinity on leaf mineral composition and salt injury symptoms of some wild grapevine (*Vitis vinifera* L. spp. *sylvestris*) genotypes. J Int Sci Vigne Vin. 2014;48:231-235.
25. Baneh DH, Hassani A, Abdollahi R. Growth and physiological responses of some wild grapevine (*Vitis vinifera* L. spp. *sylvestris*) genotypes to salinity. Bulgar J Agri Sci. 2015;21:530-535.
26. Sinclair C, Hoffmann A. Monitoring salt stress in grapevines: are measures of plant trait variability useful?. J Appl Ecol. 2003;40:928-937.
27. Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman H M. Isolation of the *Arabidopsis* ABI3 gene by positional cloning. Plant Cell. 1992;4(10):1251-1261.

28. Shi H, Zhu JK. Regulation of expression of the vacuolar $Na^+/H+$ antiporter gene AtNHX1 by salt stress and abscisic acid. Plant Mol Biol. 2002;50:543-50.
29. Cramer GR, Ergul A, Grimplet J, Tillett RL, Tattersall EA, Bohlman MC, et al. Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. Funct Integr Genomics. 2007;7(2):111-134.
30. Ohori T, Fujiyama H. Water deficit and abscisic acid production of *Salicornia bigelovii* under salinity stress. Soil Sci Plant Nut. 2011;57:566-572.