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ORIGINAL ARTICLE

Transcriptomic VvPHO1 Gene Profiles Relation with Abscisic Acid (ABA) and Salicylic Acid (SA) in Grapevine (*Vitis vinifera* L.) under Salt Stress

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Expression profile of VvPHO1 gene, abscisic acid (ABA) and salicylic acid (SA) were investigated in Baladi grapevine cultivar and B41 grapevine rootstock after different times (24, 48, 72 and 96 hours) of exposure to 2 dS/m sea water (SW). Quantitative RT-qPCR test revealed that the VvPHO1 gene, showed up-regulation from 0.27 to 2.61-fold in Baladi cv. and from 0.5 to 6.1-fold in B41 rootstock when exposure time increased from 24 to 96 h. Moreover, SW treatment caused decrease in total ABA content from 419.15 to 128 ng/g (3.274 fold) in Baladi cv. and from 1674.95 to 1559 ng/g (1.074 fold) in B41 rootstock when exposure time increased from 24 to 96 h. As for total SA, this parameter followed inverse tendency in Baladi cv. and B41 rootstock; it decreased from 126.45 to 25.6 ng/g (4.94 fold) in Baladi cv. and increased from 9.54 to 147 ng/g (15.41 fold) in B41 rootstock when exposure time increased from 24 to 96 h. Overall, data showed that VvPHO1 transcript pattern was closely related with SA level in B41 rootstock; referring that SA phytohormone could be implicated in VvPHO1 genes pathway mediates salt tolerance in grapevines.

Key words: Grapevine, VvPHO1 gene, abscisic acid (ABA), salicylic acid (SA), salt stress

Grapevine (*Vitis vinifera* L.) is grown in all countries of the world and contribute more money to farmers than the other crops. It is one of the most widely cultivated plant species of agricultural interest, and is extensively appreciated for its fruits and the wines made from its fruits. Due to very its important fruit over the world, it sometime called king of the fruit. According to the Food and Agriculture Organization (FAO), 75.866 km^2 of the world are dedicated to grapes. Approximately 71% of world grape production is used for wine, 27% as fresh fruit, and 2% as dried fruit (Khan et al., 2020). In Syria, the unique historical and geographical emplacement encouraged its cultivation for more than 5000 years (Khalil et al., 2017). Baladi cultivar is one of the most grapevine cultivars grown in Syria among the 100 distributed grapevine ones with estimated production of 20% from the total Syrian grapevine production (Bayer, 2018).

Salt stress is an abiotic stress that affect plant, growth, development and reproduction (Qiu et al., 2017; Saleh et al., 2020; Shu et al., 2022). Salt stress induced reactive oxygen species (ROS) that caused RNA, DNA, proteins, genes and others molecules perturbation. Thereby, plants developed different mechanism defenses to reduce and alleviate salt stress damages *e.g*. solutes organic (betaine, proline and ABA contents), proteins and genes accumulation (Khan et al., 2014; Qiu et al., 2017; Saleh and Alshehada, 2018a; 2018b; Saleh et al., 2020), activating ROS scavenging and enhancing photosynthesis and ion homoestasis (Qiu et al., 2017; Zhang et al., 2017).

Salt stress tolerance is a complex trait where many genes families are involved in this process *e.g*. NHX1, DREB, ABRE, CDPK, osmotin or osmotin-like protein and PHO1 (Saleh and Alshehada, 2018a; 2018b; Saleh et al., 2020). Of which, PHOSPHATE1 (PHO1) gene family plays different roles *e.g.* in stomatal responses to ABA and a possible interaction among different signal transduction pathways in plants such as drought and salinity stresses, inorganic phosphate (Pi) transfer and signal transduction, and plant development (Wang et al., 2019; Bechtaoui et al., 2021). In this regards, PHO1 expression has been evaluated under salt stress in soybean (*Glycine soja*) (Wang et al., 2019).

It has been demonstrated that phytohormones *e.g*. salicylic acid (SA), jasmonic acid (JA) and abscisic acid (ABA) have combined effects in plant development and defense (Cutler et al., 2010). They play an important role in mediating responses to different abiotic stresses; *e.g*. JA functions in different physiological manners in plants, such as the regulation of plant responses to abiotic stresses (Zhang et al., 2017); *e.g*. exogenous accumulation such as ABA and JA in *Brassica napus* L. exposed to NaCl (Qiu et al., 2017; Shu et al., 2022); JA in sweet potato (*Ipomoea batatas* L. Lam.) under NaCl (Zhang et al., 2017); ABA in *V. vinifera* under drought and salt stresses (Marusig and Tombesi, 2020); SA in salt stress tolerance of *Vicia faba* (Azooz, 2009), *Brassica juncea* (Nazar et al., 2011; 2015), *Medicago sativa* (Palma et al., 2013), and *Vigna radiata* (Khan et al., 2014); ABA and SA in grapevine (*V. vinifera* L.) under NaCl (Saleh et al., 2020).

PHO1 gene function in salt stress tolerance in plant species has been investigated. However, its function in salt stress tolerance in grapevine (*V. vinifera* L.) is not emphasized until now. Thus, our goal was to investigate ABA and SA phytohormones implication in VvPHO1 gene expression pathway mediates salt tolerance in grapevines.

MATERIALS AND METHODS

Preparation of plant material

Baladi grapevine cultivar and B41 as an introduced rootstock were obtained from the General Commission for Scientific Agricultural Research of Syria (GCSAR) providing source of multiplication. Preparation of plant materials and experimental conditions were as described by Saleh and Alshehada (2018a).

Grapevine plants were irrigated with tap water twice per week for two months prior to salt stress application. They were divided into two groups; the first group was continuously irrigated with tap water as a control. Whereas, the other one was treated with 2 dS/m sea water (SW). Leaf samples (for control and stressed plants) were collected 24, 48, 72 and 96 h after salt treatment and were kept frozen in liquid nitrogen.

Isolation of RNA and cDNA synthesis

RNA extraction and cDNA synthesis were performed as reported by Saleh and Alshehada (2018a). Synthesized cDNA was kept at −20 °C until use.

Quantitative real-time PCR (qPCR) test. Expression profile of VvPHO1 gene was investigated in Baladdi grapevine cultivar and B41 grapevine rootstock after different times of exposure to 2 dS/m SW. Quantitative RT-qPCR test was performed by using the VvPHO1 gene [(*V. vinifera* phosphate (Pi) transporter 1] 5′-GCATTGGTCTATTGTGGGGTCC-3′ (VvPHO1F) and 5′-GTGAAAAACAGGCACTGATTGACC-3′ (VvPHO1R). Whereas, the VvEF1α gene was chosen as a housekeeping gene and amplified with the VvEF1αF (5′-CGGGCAAGAGATACCTCAAT-3′) and the VvEF1αR (5′-AGAGCCTCTCCCTCAAAAGG -3′) primers.

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 performed as described by Saleh and Alshehada (2018a).

ABA and SA determination

Leaf samples (for control and stressed plants) were collected after 24, 48, 72 and 96 h after SW treatment. Approximately 200 mg of freshly ground leaves following the method of Trapp et al. (2014), with minor modifications. Samples were kept frozen at −42 °C overnight. 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 for 10 min at 16000 g/4 °C. To quantify of ABA and SA, high-performance liquid chromatography coupled mass spectrophotometer (HPLCMS/MS) system (Agilent Technologies, Böblingen, Germany) was employed. 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 method.

RESULTS AND DISCUSSION

Expression patterns of VvPHO1 gene has been evaluated after various periods of time (24, 48, 72 and 96 h) in leaves of Baladi cultivar and B41 rootstock grapevine under 2 dS/m SW based on RT-qPCR technique. Data revealed that the VvPHO1 gene, showed up-regulation from 0.27 to 2.61-fold when exposure time increased from 24 to 96 h in Baladi cv. Whereas, it showed up-regulation from 0.5 to 6.1-fold when exposure time increased from 24 to 96 h in B41 rootstock (Figure 1).

Overall, VvPHO1 transcript expression was higher in B41 rootstock compared to Baladi grapevine cv. Similarly, Saleh and Alshehada (2018a) reported that the VvOSM1 transcript expression was higher in B41 rootstock compared to Baladi grapevine cv. after 3 days exposure to salt stress of 2 dS/m SW. Whereas, Saleh and Alshehada (2018b) reported up-regulation of VvABF1 and VvAREB2 transcripts in Baladi and Halawani grapevine 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. Moreover, VvNHX1, VvABF1, VvCBF4 and VvOSM1 genes were up-regulated in higher level in B41 rootstock compared to Baladi grapevine cv. after 3 days exposure to salt stress of 2 dS/m SW (Saleh et al., 2020).

SW treatment caused decrease in total ABA content (Figure 2), from 419.15 to 128 ng/g (3.274 fold) in Baladi cv. and from 1674.95 to 1559 ng/g (1.074 fold) in B41 rootstock when exposure time increased from 24 to 96 h. This decrease positively related with prolonged exposure time from when exposure time increased from 24 to 96 h.

Zhang et al. (2017) reported 8,744 and 10,413 DEGs (differentially expressed genes) in Lizixiang (salt-sensitive variety) and line ND98 (salt-tolerant), respectively, were involved in sweet potato root exposed to 200 mM NaCl. Moreover, they reported that the salt tolerant ND98 line has been characterized by significant up-regulation genes involved in the jasmonic acid (JA) biosynthesis and signalling pathways and ion transport, more accumulation of JA, a higher degree of stomatal closure and a lower level of Na^+ compared to Lizixiang salt-sensitive variety;

referring that the JA signalling pathway plays an important role in the response of sweet potato to salt stress. As for ABA biosynthesis genes zeaxanthin epoxidase (*IbZEP*) and 9-cis-epoxycarotenoid dioxygenase (*IbNCED*) genes, showed change expression level under salt stress in both studied genotypes, but these genes were not induced in ND98 under salt stress. Similarly, The SA biosynthesis gene phenylalanine ammonia-lyase (*IbPAL*) was up-regulated, but other biosynthesis and signalling genes were not induced under salt stress in ND98. The expression patterns of these genes were consistent with the results of the RNA-Seq analysis. Whereas, Qiu et al. (2017) reported up-regulated 740 DEGs genes along with 495 down-regulated genes were identified in *Brassica napus* L. after 12 h of 200 mM NaCl treatment. Moreover, they reported a relationship between DEGs and signal transduction, osmolyte synthesis, transcription factors, and antioxidant proteins. Moreover, Wang et al. (2019) reported that the GsPHO1 gene displayed relatively different expression patterns under salt stress between Suinong 14 (SN14) and ZYD00006 (ZYD6) soybean (*Glycine soja*) cultivars; and it enhanced salt stress tolerance in soybean at the high salt concentration (200 mM NaCl).

More recently, Shu et al. (2022) reported significant increase in gene expression of BnHSPs, BnCAT2, BnWRKY40, BnMYC, BnJAZ [belonged to differentially expressed genes (DEGs) group], and others in *Brassica napus* L. leaves under 24 h salt stress using quantitative RT-PCR analysis. Moreover, they reported that JA and ABA play an important role in alleviating salt stress damages. Also, they reported closed relationship between L-cysteine and *Brassica napus* salt tolerance revealed that L-cysteine might be used as a molecular marker. Furthermore, they reported that the expression of ABA related DEGs were also increased by salt stress, *e.g*. BnPYL8, BnPUB9, BnCIPK15, BnABI1, BnNAC002, BnSRK2I, etc.

The PHO1 gene is expressed as well to improve survival of plants under Pi stress and to regulate the transduction of the ABA signal under salt stress (Bechtaoui et al., 2021).

As for total SA, its alteration content (Figure 3); where, this parameter followed inverse tendency in Baladi cv. and B41 rootstock when exposure time prolonged from 24 to 96 h. in this regards, it decreased from 126.45 to 25.6 ng/g (4.94 fold) in Baladi cv. and increased from 9.54 to 147 ng/g (15.41 fold) in B41 rootstock when exposure time increased from 24 to 96 h.

Data showed that VvPHO1 transcript pattern was closely related with SA level in B41 rootstock; referring that SA phytohormone could be implicated in VvPHO1 gene pathway mediates salt tolerance in grapevines.

From the data presented herein, VvPHO1 transcript pattern was closely related with SA level in B41 grapevine rootstock under 2 dS/m SW. Similarly, Saleh et al. (2020) reported that VvAREB2 and VvABF1 transcript pattern was closely related with activation of SA level in B41 grapevine rootstock; whereas, VvOSM1 transcript pattern was inversely related with SA level in Baladi cv. under 2 dS/m SW.

Many studies showed that the SA phytohormone plays a significant role in enhancing plants stress-tolerance in different plants crops (Khan et al., 2015) *e.g*. in *V. faba* (Azooz, 2009), *B. juncea* (Nazar et al., 2011; 2015), *M. sativa* (Palma et al., 2013), and *V. radiata* (Khan et al., 2014). The latter researches suggested that the positive role of SA in salt stress tolerance could be manifested through increasing antioxidant metabolism (Nazar et al., 2011; Palma et al., 2013; Khan et al., 2014); or glycinebetaine (GB) accumulation through increased methionine (Met) and suppresses ethylene formation leading thereby to enhance antioxidant system (Khan et al., 2014); or through increase of ascorbate-glutathione enzymes pathway suggesting that SA may participate in the redox balance under salt stress (Nazar et al., 2015).

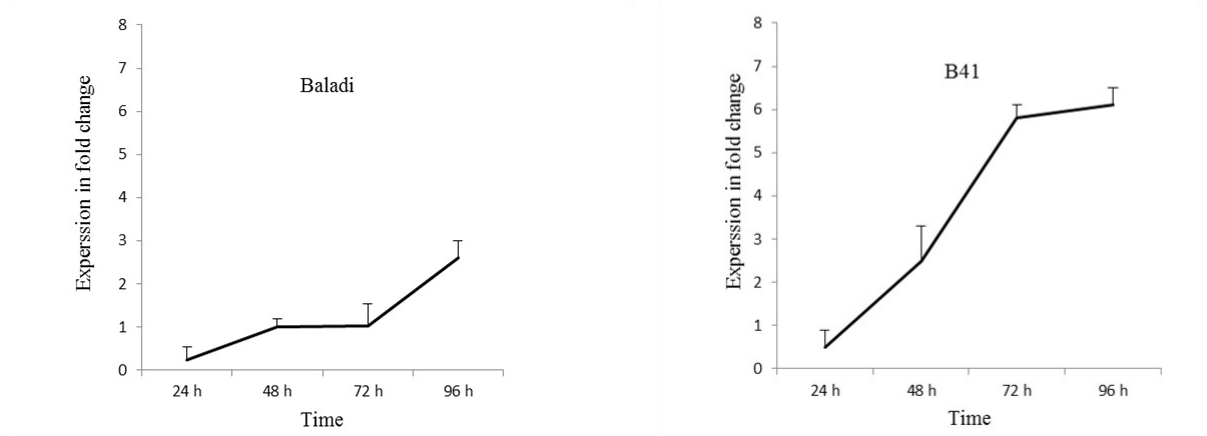

Figure. 1. Relative expression profile of VvPHO1 gene in Baladi cultivar and B41 rootstock grapevine leaves after 24, 48, 72 and 96 h of exposure to 2 dS/m SW. Error bars are representative of the standard error (Mean ± SD, n = 3).

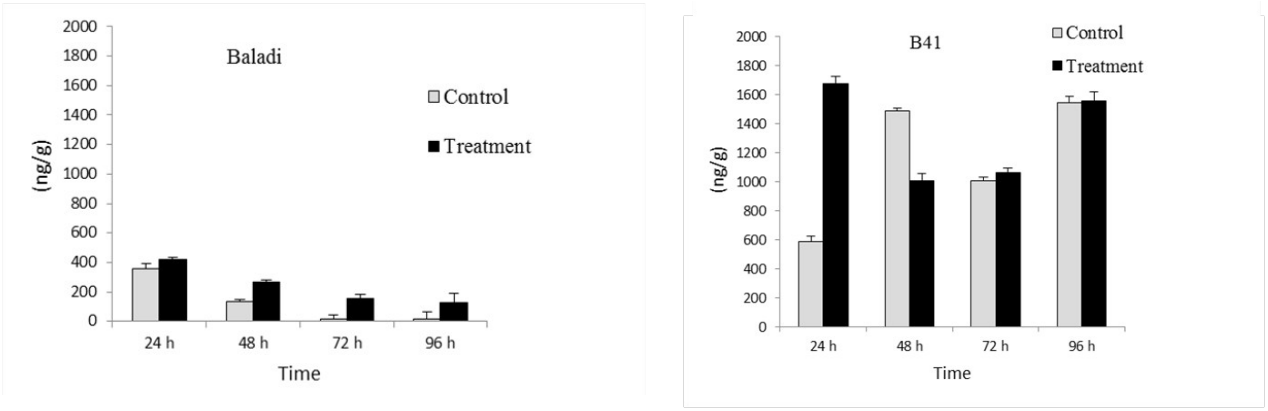

Figure 2. Total abscisic acid (ABA) content in Baladi cultivar and B41 rootstock grapevine leaves after 24, 48, 72 and 96 h of exposure to 2 dS/m SW. Error bars represent the standard error of the means (n=3).

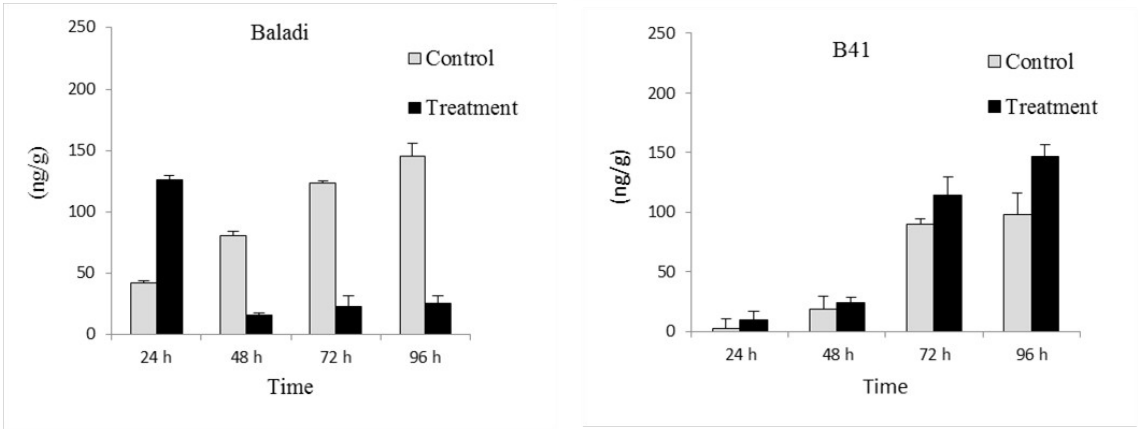

Figure 3. Total salicylic acid (SA) content in Baladi cultivar and B41 rootstock grapevine leaves after 24, 48,72 and 96 h of exposure to 2 dS/m SW. Error bars represent the standard error of the means (n=3).

CONCLUSION

Transcriptomic VvPHO1 gene profiles, abscisic acid (ABA) and salicylic acid (SA) were investigated in Baladi grapevine cv. and B41 grapevine rootstock after different times (24, 48, 72 and 96 h) of exposure to 2 dS/m SW. Quantitative RT-qPCR test revealed that the VvPHO1 gene showed higher up-regulation level in B41 rootstock compared to Baladi grapevines cv. Salt stress caused less decrease in ABA content pronounced in Baladi cv. compared to B41 rootstock. However, SA content showed inverse tendency, where, it decreased in Baladi cv. and significantly increased in B41 rootstock. Overall, VvPHO1 transcript pattern was closely related with SA level in B41 rootstock; referring that SA phytohormone could be implicated in VvPHO1 gene pathway mediates salt tolerance in grapevines. Thereby, implementation of other phytohormones in VvPHO1 gene transcript pattern in grapevine and other plants species is requested in future.

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CONFLICT OF INTERESTS

The authors declared no conflicts of interests or publication of this article.

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