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Adaptive Mechanisms During the Recovery of Tolerant and Sensitive Local Grape Genotypes Subjected to Salt Stress

Mecanismos adaptativos durante la recuperación de genotipos de uvas locales tolerantes y sensibles sometidos a estrés salino

Mecanismos adaptativos durante a recuperação de genótipos de uva locais tolerantes e sensíveis submetidos ao estresse salino

Rania Mahmoud[1*]
Fouad Ashry[2]
Roqaya Shallan[2]

[1]Deciduous Fruits Research Department, Biotechnology Central Lab, Horticulture Research Institute (HRI), Agricultural Research Center (ARC), Egypt.
[2]Faculty of Biotechnology, October University for Modern Sciences and Arts, Egypt.

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Abstract

Utilization of distinct genetic resources is an auspicious prospective strategy to combat adverse impacts of salinity, which is expected to get worse under climate change conditions, for maintaining grape production and quality. This research aims to study the adaptive mechanisms during the recovery of tolerant and sensitive salt-stressed local grape genotypes on the bases of biochemical, anatomical and gene expression responses. Transplants of three Egyptian grapes (*Vitis vinifera*); Baltim Eswid, Romy Ahmer and Romy Abiad, were exposed to sodium chloride-induced salt stress of 2.28 and 3.75 mS compared to 695 µS water-irrigated control for two months, then all plants were irrigated with tap water for additional one month for recovery. Recovered Baltim Eswid cultivar following the highest saline treatment gave maximum survival percentage (100 %), while Romy Abiad recorded the lowest rate (40 %). Suggested adaptive mechanisms include: damage reduction caused by salinity-related oxidative stress, osmotic adjustment, and perform structural modifications that allow protection. It was concluded that, Blatim Eswid is a superior salt-tolerant local grape genotype, while Romy Abiad is the most sensitive as affected mostly by oxidative stress represented by a significant increment of hydrogen peroxide content.

*Corresponding author:raaniaahmed@arc.sci.eg

Resumen

La utilización de distintos recursos genéticos es una estrategia prospectiva auspiciosa para contrarestar los impactos adversos de la salinidad, que se espera que empeore bajo las condiciones del cambio climático, para mantener la producción y la calidad de la uva. Esta investigación tiene como objetivo estudiar los mecanismos adaptativos durante la recuperación de genotipos de uva locales tolerantes y sensibles al estrés salino sobre bases de respuestas bioquímicas, anatómicas y de expresión genética. Trasplantes de tres uvas egipcias (*Vitis vinifera*); Baltim Eswid, Romy Ahmer y Romy Abiad fueron expuestos a un estrés salino inducido por cloruro de sodio de 2,28 y 3,75 mS en comparación con el control regado con agua a 695 µS durante dos meses, luego todas las plantas se irrigaron con agua del grifo durante un mes adicional para su recuperación. El cultivar Baltim Eswid recuperado después del tratamiento salino más alto dio el porcentaje máximo de supervivencia (100 %), mientras que Romy Abiad registró la tasa más baja (40 %). Los mecanismos adaptativos sugeridos incluyen: reducción de daños causados por estrés oxidativo relacionado con la salinidad, ajuste osmótico y realizar modificaciones estructurales que permitan la protección. Se concluyó que Blatim Eswid es un genotipo de uva local superior tolerante a la sal, mientras que Romy Abiad es el más sensible ya que se ve afectado principalmente por el estrés oxidativo representado por un incremento significativo del contenido de peróxido de hidrógeno.

Palabras clave: uva, salinidad, recuperación, genotipos.

Resumo

A utilização de recursos genéticos distintos é uma estratégia prospectiva auspiciosa para combater os impactos adversos da salinidade, que deverá piorar sob as condições das alterações climáticas, para manter a produção e a qualidade da uva. A pesquisa atual visa estudar os mecanismos adaptativos durante a recuperação de genótipos de uva locais tolerantes e sensíveis ao estresse salino com base em respostas bioquímicas, anatômicas e de expressão gênica. Transplantes de raiz própria de três uvas egípcias (*Vitis vinifera*); Baltim Eswid, Romy Ahmer e Romy Abiad, foram expostos ao estresse salino induzido por cloreto de sódio de 2,28 e 3,75 mS em comparação com o controle irrigado com água de 695 µS por dois meses, depois todas as plantas foram irrigadas com água da torneira por mais um mês para recuperação. A cultivar Baltim Eswid recuperada após o tratamento salino mais elevado proporcionou a percentagem máxima de sobrevivência (100 %), enquanto Romy Abiad registou a taxa mais baixa (40 %). Os mecanismos adaptativos sugeridos incluem: redução de danos causados pelo estresse oxidativo relacionado à salinidade, ajuste osmótico e realização de modificações estruturais que permitam proteção. Concluiu-se que Blatim Eswid é um genótipo de uva local tolerante ao sal superior, enquanto Romy Abiad é o mais sensível, pois é afetado principalmente pelo estresse oxidativo representado por um aumento significativo no teor de peróxido de hidrogênio.

Palavras-chave: uva, salinidade, recuperação, genótipos.

Introduction

Grapes (*Vitis vinifera* L.) are one of the most widespread fruit crops worldwide. In 2017, Mirás-Avalos and Intrigliolo noted that climate change imposes constraints on grape production, which are expected to worsen with rising temperatures, less precipitation, and more frequent heatwaves. Anyway, the most insistent challenges facing the grapevine, especially in arid and semi-arid regions, are the increased risk of drought and salinity due to high evaporation rate and water scarcity. Considering the fact that, diversity of *Vitis* spp. genetic resources is certainly a main factor that reinforced the historical prevalence of grapevine cultivation around the world, so selecting the most proper plant materials is the principle underlying long-term resilience strategies for viticulture under the influence of a changing climate (Pastore *et al*., 2022). There is a limited number of publications that have focused on evaluating comparative salt tolerance and recovery traits among diverse grape genotypes, especially local ones, which is important both for adequate cultivars selection, as well as for improving and developing superior grapevines through breeding efforts. Accordingly, current research aims to study the adaptive mechanisms during recovery of tolerant and sensitive salt-stressed local grape genotypes on bases of biochemical, anatomical and gene expression responses.

Materials and methods

Plant materials and experimental design

A pot culture experiment was done under shade net house conditions at Horticulture Research Institute experimental orchard. The experiment was repeated twice during 2022 and 2023 successive seasons to confirm the results obtained. Three Egyptian genotypes of grapes (*Vitis vinifera* L.) were selected for evaluation: Baltim Eswid which is a blue-black grape traditionally grown on the hillsides of Egypt's northern coast under rainfed conditions, Romy Ahmer red grape as one of the most common local cultivars, and Romy Abiad white-green grape that is classified as salt sensitive genotype based on morpho-physiological responses (Mahmoud *et al*., 2023). Six months old own rooted healthy transplants of the three tested genotypes, planted in polyethylene bags filled by 1:2 (v/v) mixture of peatmoss and washed sand, were exposed to sodium chloride-induced salt stress of 2.28 mS (low salt treatment) and 3.75 mS (high salt treatment) compared to 695 µS tap water-irrigated control for two months (Mahmoud *et al*., 2023), then all plants were irrigated with tap water (695 µS) for another month to recover. A complete randomized blocks design was applied for arranging experimental treatments. Three replicates were sampled from each treatment, and each replicate included four symmetrical transplants. Survival rate (%) was calculated as a percent of survivors count versus the total number of treated plants, thereafter, leaf samples were collected to perform the required analysis. Leaf samples of seedlings that recovered after being exposed to the highest level of salt stress were subjected to anatomical and gene expression analysis compared to the corresponding control for each genotype.

Biochemical analysis

Content of total phenols was measured with Folin-Ciocalteu's reagent (Singleton & Rossi, 1965). The ferric reducing antioxidant power (FRAP) assay (Quisumbing, 1978) was applied for determination of total antioxidant activity using ascorbic acid as reference standard for calculation. Fresh leaf samples were subjected to hydrogen peroxide (H_2O_2) quantification as described by Velikova *et al*. (2000). The reducing sugars content was determined using the 3,5-dinitrosalicylic acid (DNSA) method (Miller, 1959). Quantitative amino acids content was determined using the ninhydrin reaction (Kowalska *et al*., 2022).

Leaves anatomical structure

Specimens were separated from the midrib region of the 4th upper leaf on the main stem (Mahmoud *et al*., 2023). Anatomical analysis following microtechnique procedures described by Nassar and El-Sahhar (1998) were carried out at the Cairo University Research Park, Faculty of Agriculture.

Gene expression analysis

Collected leaf samples were immediately frozen in liquid nitrogen, then kept at -80°C for extraction of total RNA as explained by Mahmoud *et al*. (2023). Differential expression of two targeted genes; VvChS & VvEDS1, and VvEF1-α as a reference gene (Mohammadkhani *et al*., 2016) was confirmed by qPCR. Reverse transcription was performed with "Thermo Scientific" RevertAid First Strand cDNA Synthesis Kit. "Willowfort" *HERA*^{PLUS} SYBR® Green qPCR Kit was used for all assays. Thermal profile was set as; 95 °C for 10 min, forty repeated cycles; 95 °C for 15 s, 58 °C for 1 min, and 72 °C for 20 s, finally a climb in increments of 0.05 °C from 58 to 95 °C for the high-resolution melting curve (Buesa *et al*., 2022).

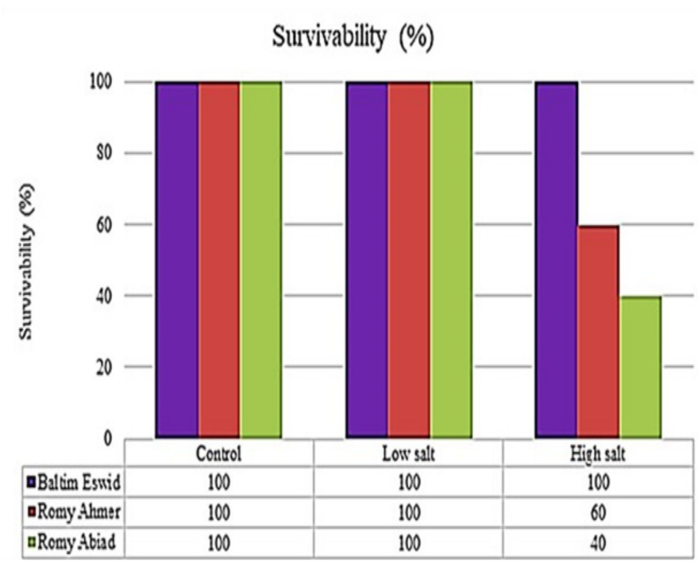
Statistical analysis

Data were ANOVA statistically analysed as factorial randomized block design and significant variations were specified by obtained values of L.S.D. at 0.05 (Snedecor & Cochran, 1989). The results listed represent mean values of the two conducted experiments where data of collected samples for both seasons were remarkably similar.

Results and discussion

Survivability

Low salt pretreatment (2.28 mS) had no effect on survival percentage of grape seedlings during recovery (figure 1). The high salt pretreatment (3.75 mS) significantly decreased recovered grape seedlings survivability of Romy Ahmer and Romy Abiad recording 60 and 40 %, respectively, while Baltim Eswid maintained the maximum survivability of 100 %.



LSD 0.05 : Cultivars & Treatments = 8.81 , Interaction = 15.26

Figure 1. Survivability (%) of recovered local grape genotypes following salt treatments.

Often the response of growth to salinity is considered a basic assessment of how tolerant genotype is, which reflects on its performance during recovery. Mahmoud *et al*. (2023) evaluated nine grapevine varieties against salinity where Romy Abiad was categorized as the most sensitive variety. Based on morpho-physiological responses of the three tested local grape genotypes under stress conditions, Romy Abiad was the least regarding leaves count, moisture and relative water contents, while Romy Ahmer recorded the highest root electrolyte leakage as affected by salt, with no significant effect on survivability up to 2,000 ppm NaCl for all cultivars (Mahmoud *et al*., 2023). However, survival percentage during recovery notably varied according to the cultivars under study indicating limited ability of Romy Ahmer to recover after being exposed to salt stress comparing to Baltim Eswid genotype which is the most relatively salt tolerant. Baltim Eswid cultivar is a local grapevine, that is being planted long time ago on the hill side of the north coast of Egypt specifically in Baltim city, Kafr El-Sheikh governorate, which is now on the verge of extinction.

Biochemical responses

Antioxidant potential

Antioxidant potential of leaves, represented in content of total phenols and quantified overall antioxidant activity, was reduced after recovery following high salt treatment with the lowest mean values of 8.64 and 7.25 mg.g^{-1}, respectively (table 1). Generally, recovered Romy Abiad genotype scored relatively lowest values of both total phenols content and total antioxidant activity as compared with other tested cultivars.

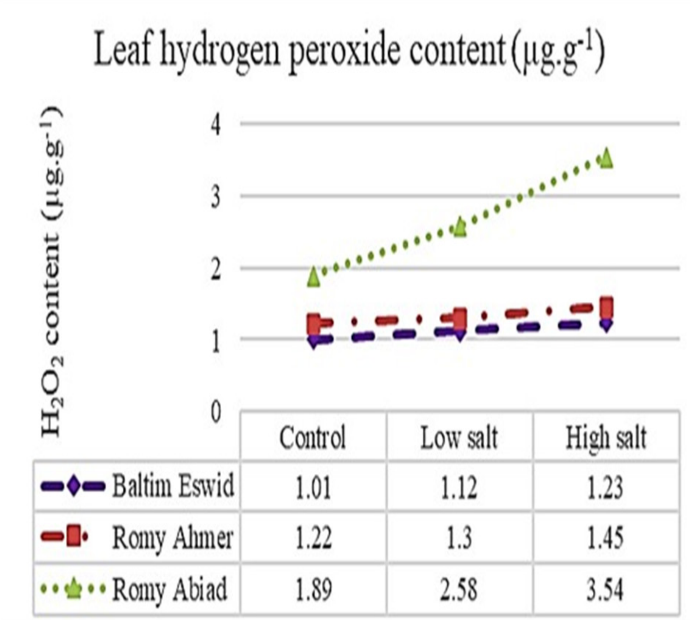
One of the key components associated with salt stress and eventual damage is reactive oxygen species (ROS). Hence, synthesis of antioxidants is another focal point of salt tolerance, which is a crucial mechanism for ROS detoxification in grapevine (Mohammadkhani & Abbaspour, 2017). Many phenolic compounds are stress-induced metabolites in plants. Excessive accumulation of phenolics has been reported to prevents cellular oxidative rupture by improving radical scavenging activity. Soluble phenols contain electron-donating mediators that provide their antioxidant capabilities, thus mitigating additional ROS accumulation. This overproduction is likely stimulated by promoting the phenylpropanoid pathway and enhancing phenyl-aminolyase gene expression (El-Banna *et al.,* 2022).

Oxidative stress indicator (H_2O_2)

Leaf H_2O_2 content of recovered seedlings increased gradually as affected by increasing level of salt stress pretreatment (figure 2). Romy Abiad genotype scored significantly highest content of H_2O_2 (2.67 µg.g^{-1} in average). Hydrogen peroxide (H_2O_2) is a relatively long-lived molecule of reactive oxygen species (ROS) that is enhanced in response to osmotic and ionic stresses. Mohammadkhani *et al*. (2016) demonstrated that, low ROS level acts as an elicitor or hormone-like substance with easy diffusion properties that plays a significant role in signal transduction pathways stimulating systemic responses in plants to salt stress which may aid salinity tolerance, while an excessive ROS content, as for Romy Abiad genotype, could cause oxidative damage and degradation of biopolymers including cell wall polysaccharides and nucleic acids.

Table 1. Leaf antioxidant capacity (mg.g⁻¹) of recovered local grape genotypes following salt treatments.

	Total phenolic content				Total antioxidant activity			
	Control	Low salt	High salt	Mean	Control	Low salt	High salt	Mean
Baltim Eswid	9.47	10.56	10.16	10.06	7.53	9.33	8.41	8.42
Romy Ahmer	10.38	9.57	8.52	9.49	10.13	9.67	7.22	9.01
Romy Abiad	8.83	8.69	7.25	8.26	7.66	7.52	6.13	7.10
Mean	9.56	9.61	8.64		8.44	8.84	7.25	
LSD 0.05	Cultivars & Treatments = 0.70 Interaction = 1.21				Cultivars & Treatments = 0.97 Interaction = 1.68			



LSD 0.05 : Cultivars & Treatments = 0.27 , Interaction = 0.47

Figure 2. Leaf hydrogen peroxide content (µg.g⁻¹) of recovered local grape genotypes following salt treatments.

Osmotic adjustment

Osmotic adjustment was expressed in leaf contents of reducing sugars and total free amino acids (table 2). Upon recovery, the highest leaf reducing sugars content mean value (2.73 g.100 g^{-1}) was recorded as affected by low salt pretreatment, while the lowest leaf total free amino acids content mean value (0.09 g.100 g^{-1}) was observed as affected by high salt pretreatment. However, Romy Abiad genotype attained lowest contents of both reducing sugars and total free amino acids (2.36 and 0.10 g.100 g^{-1} in average, respectively).

The mechanism of osmotic adjustment is a strategy that could help plants to avoid ion toxicity and maintain water uptake under saline conditions. To preserve osmotic homeostasis, plants secrete osmolytes and osmoprotectants as essential abiotic stress alleviators to encounter harsh environmental conditions by lowering or balancing the osmotic potential of intracellular and extracellular ions. There are some low-molecular-weight, nontoxic compounds that accumulate in plants in response to drought and salinity stress without interfering with normal metabolism. Soluble sugars such as sucrose, trehalose, and sugar alcohols and other osmolytes such as glycine betaine and proline amino acid act as the osmoprotectants. Soluble sugars play an important role in maintaining cellular organization and photosynthesis efficiency, and detoxification of reactive oxygen species (ROS) by acting as metabolic signals in response to several abiotic stresses. Combined together, they protect plants by exercising many physiological responses, such as strengthening membrane integrity, regulating antioxidant enzymatic activity, and fulfilling water requirements under stress conditions (Roychoudhury & Tripathi, 2020).

Leaves anatomical structure

Most measured leaf anatomical characteristics of recovered Romy Abiad genotype were adversely affected by severe salt stress, except for thickness of palisade chlorenchyma tissue which is the principal site of photosynthesis as it contains numerous chloroplasts (table 3 & figures 3, 4).

Aside from biochemical responses, plants can modify leaf's anatomical structure along with altering morphology to adapt under saline conditions (Mahmoud *et al*., 2023). Since preventing water loss is a crucial adaptive mechanism versus salt stress, thick epidermis is a characteristic of many plant varieties that are tolerant to salt, helping to avoid dehydration associated with salinity (Ashraf *et al*., 2010). In accordance, the most tolerant genotype "Baltim Eswid" could maintain thicky of both lower and upper epidermis under conditions of high salt stress, while both Romy Ahmer and Romy Abiad got thinner lower epidermis.

Table 2. Leaf osmolytes content (g.100 g⁻¹) of recovered local grape genotypes following salt treatments.

	Reducing sugars content				Total free amino acids content			
	Control	Low salt	High salt	Mean	Control	Low salt	High salt	Mean
Baltim Eswid	2.61	2.87	2.58	2.68	0.12	0.13	0.10	0.12
Romy Ahmer	2.55	2.85	2.12	2.51	0.13	0.13	0.09	0.12
Romy Abiad	2.25	2.47	2.35	2.36	0.11	0.11	0.08	0.10
Mean	2.47	2.73	2.35		0.12	0.12	0.09	
LSD 0.05	Cultivars & Treatments = 0.15 Interaction = 0.27				Cultivars & Treatments = 0.01 Interaction = 0.02			

Table 3. Relative changes (%) in measured leaf anatomical traits of recovered local grape genotypes compared to control.

Anatomical features	Baltim Eswid	Romy Ahmer	Romy Abiad
Upper Epidermis thickness	+46.3	+49.6	-4.7
Lower Epidermis thickness	+20.4	-23.4	-15.8
Palisade Mesophyll thickness	+42.2	+25.5	+44.3
Spongy Mesophyll thickness	+40.3	+32.1	-15.4
Xylem Tissue thickness	+17.9	+84.9	-3.6
Phloem Tissue thickness	+43.8	+22.7	-22.4
Midvein thickness	+27.6	+44.0	-2.14
Xylem Vessel diameter	+18.1	0	-11.9

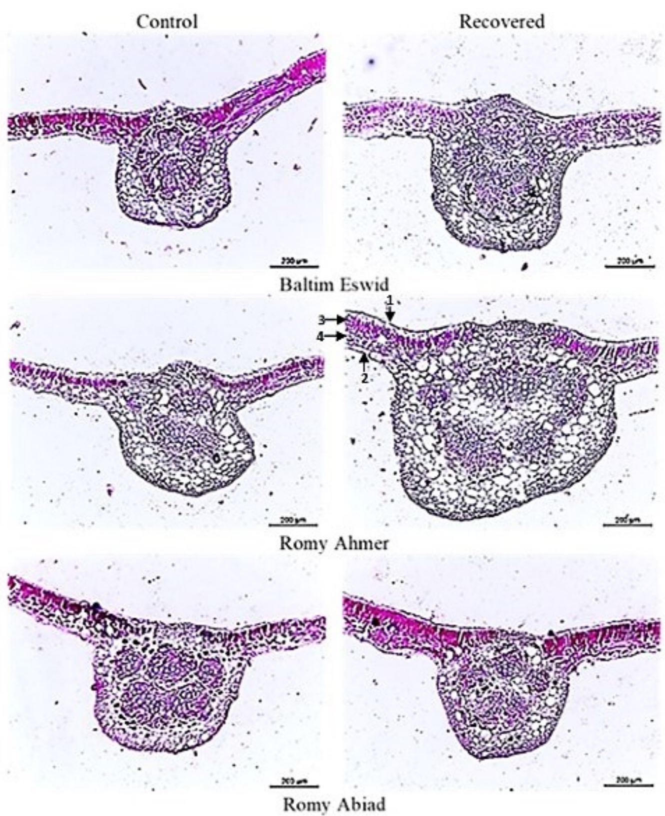


Figure 3. Transverse sections through the leaf blade of recovered local grape genotypes comparing to control. In the figure: 1: Upper Epidermis; 2: Lower Epidermis; 3: Palisade Mesophyll; 4: Spongy Mesophyll.

The reduced thickness of epidermis may be due to restricted cell division with increased salt level (Parida *et al*., 2016). Otherwise, thicker epidermis may aid better adaptation under elevated salt stress by keeping a rate of transpiration that maintains moisture of the mesophyll tissue and improve the efficiency water usage, also offers extra area for efficacious segregation of sodium in the epidermis of leaves. Additionally, enhanced thickness of the spongy mesophyll in recovered Baltim Eswid and Romy Ahmer genotypes may aid maintain moisture and turgor of leaves (Parida *et al*., 2016).

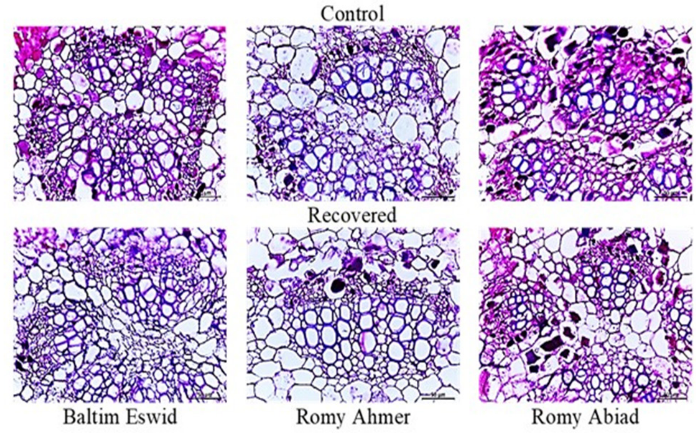


Figure 4. Xylem vessels transverse sections through the leaf blade of recovered local grape genotypes comparing to control.

Also, diameter of conductive xylem vessels was enhanced in Baltim Eswid during recovery while maintained stable in Romy Ahmer, which is functionally related to holding of various elements. A reduction in xylem vessel diameter and xylem tissue thickness was observed in the recovered Romy Abiad genotype, indicating that there was no considerable increase in xylem vessel density as well. On the other hand, xylem tissue thickness was increased in the recovered Romy Ahmer genotype which indicates enhanced xylem vessel density where constant xylem vessel diameter was observed. Modifications of vessel size are likely to be a common mechanism of response to water stress in a large-vessel species such as the grapevine (Lovisolo & Schubert, 1998). Reduced development of xylem vessels in grapevines exposed to moderate water stress may contribute to controlling water flow and reducing vulnerability to xylem embolism, which helps maintain hydraulic conductivity at low water potential, thus increasing the integrity of xylem if compensated by an increase in vessel density.

Gene expression analysis

Both VvChS and EDS1 genes were over-expressed in leaves of Romy Ahmer and Romy Abiad genotypes during recovery following the highest saline treatment compared to control, while down-regulated in Baltim Eswid cultivar (figure 5).

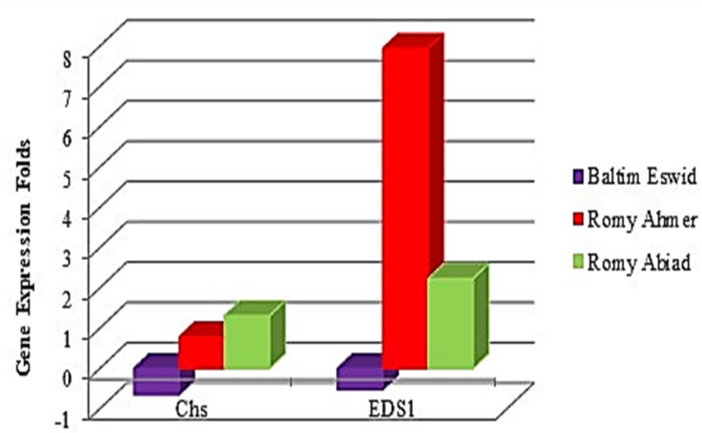


Figure 5. Changes in expression level of targeted genes for recovered local grape genotypes relatively to control. In the figure: ChS: Chalcone synthase; EDS1: ENHANCED DISEASE SUSCEPTIBILITY 1.

A key step to interpret genome function in response to various environmental signals is to determine the pattern of how gene expression is regulated. Upon exposure to stress, plants can express several genes as a defense response. Synthase of chalcone (ChS), that is a main enzyme in biosynthesis of flavonoids which intrinsic in the pathway of phenylpropanoid, is commonly induced in different species of plants under abiotic stress conditions (Mohammadkhani *et al*., 2016). It is possibly that, salt-affected Romy Ahmer and Romy Abiad genotypes that suffer during recovery attempt to stimulate the mechanisms of defense by rising the VvChS transcription. For VvEDS1, more studies should be done to specify its exact function in grapevine defense mechanism, in which agrees with Chong *et al*. (2008). EDS1 is a key regulator of cell death in the hypersensitive response to stress signals. Plants responses to stress can be induced by ROS perception as a signal which promotes the program of genetic response to stress, where protein of EDS1 appears to be related to controlling the singlet-oxygen–mediated visible responses to stress (Mohammadkhani *et al*., 2016). It was hypothesized that, EDS1 may regulate plant recovery after being exposed to ecological stress conditions.

Conclusion

Recovery is one of stress tolerance mechanisms in plants indicating the ability to restore metabolism after cessation of severe stress conditions. Basic studies on stress tolerance in plants have generally focused on their responses under different applied stress treatments, but very little is known about the equally important stress recovery mechanisms, which are essential to ensure sustainable crop production under intermittent stress events. The objectives of this research were to evaluate the salt tolerance and recovery attributes of three native grape genotypes (*Vitis vinifera*); Baltim Eswid, Romy Ahmer and Romy Abiad, on the bases of biochemical, anatomical and gene expression responses. Suggested adaptive mechanisms include: damage reduction caused by salinity-related oxidative stress, osmotic adjustment, and perform structural modifications that allow protection. It was concluded that, Blatim Eswid is a superior salt-tolerant local grape genotype, while Romy Abiad is the most sensitive as affected mostly by oxidative stress. The study highlighted the paramount importance of preserving Egyptian table grape germplasm, especially those that are threatened with extinction, as they adapt to harsh ecological conditions and neglected cultural managements, which can aid countering the expected constraints associated with climate change on viticulture and adapt under adverse conditions to ensure maintaining productivity and quality of grapes.

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