Salinity Depression *in Vitro* Impress the Growth Potential and, Mediates the Antioxidants Pool and the Tolerance Genes Expression of Grapevine

Mohammad Aazami (aazami58@gmail.com)
University of Maragheh
Mohammad Hassanpouraghdam
University of Maragheh

Research Article

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Abstract

Undoubtedly, salinity is the major environmental stress affecting the crops growth, development and yield. Grapevine is a dominant horticulture crop and mildly sensitive to the salinity. Salinity triggers variations at the cellular and molecular levels and hence induces some specific genes expression. The responses of two grapevine cultivars (Fakhri and Sultanin) were evaluated for its tolerance to the salinity stress *in vitro*. The results for the explants exposed to the salinity revealed that the viability, fresh weight of the regenerated explant and the proliferation rate were declined compared to the control ones. The activity of SOD enzyme and MDA content were increased with salinity. However, protein content declined. There was no significant difference in CAT and APX activities with the salinity treatment. With salinity adding up, the *DREB/CBFs* genes expression pattern was significantly increased in both cultivars. 'Fakhri' was more responsive in growth parameters and the activity of antioxidant enzymes and higher expression rate of *DREB/CBFs* under salinity compared to the variety 'Sultanin'.

Intruduction

Salinity is of the predominant environmental stress factors greatly reduce the growth potential, water availability, yield and quality as well as devastates the ecological biomes and is an eminent treat for the human food security[1].

Salinity imposes osmotic and ionic pressures on plants[2]. Osmotic stress is the consequence of the reduced water potential and, the ionic stress is the result of hyper-absorption and accumulation of Na^+ and Cl^- ions in the saline media[3]. Plants tolerant to the salinity depression are able to coagulate the toxic saline ions in the vacuoles to prevent their toxic concentrations on cell metabolism[4]. With high Na^+ concentrations, the absorption of Ca^{2+}, K^+ and Mg^{2+} greatly reduces. Moreover, excess Cl^- availability restricts the nitrate accessibility to the plants[2].

The diverse grapevine cultivars are classified as semi-sensitive to the salinity[5]. The salinity impacts on grapevines are including the diminished CO_2 fixation, declined dry matter accumulation and a great loss in the fruit bunches number, berry volume and size and, the prominent growth and yield reductions. Salinity furthermore, alters the berry composition by increasing Cl^- and Na^+ concentration, and reducing sugar content and higher acidity[6]. Salinity damage on grape is dependent upon the saline ions concentration, plant growth stage, cultivar type and the scion: rootstock combination[7,8].

A common response of many plants to the salinity is the over-production of a bunch of organic compounds to ameliorate its deleterious effects. These compounds are carbohydrates, amino acids and proteins, which act as osmolytes to combat the stress[9,10]. Furthermore, plants with the dynamic antioxidant enzymes pool are able to quieten damages caused by the environmental stress factors. The efficient antioxidant system devotes reasonable tolerance to the environmental stress and salinity[11,12]. Antioxidant system is composed of compounds like ascorbate, glutathione, carotenoid plus ROS scavenging enzymes; Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX), Guaiacol peroxidases (GPX), Glutathione reductase (GR) and some others[13]. Osmolytes protect the plants against stressors by their association in cell water potential, toxic compounds detoxification, ROS molecules scavenging, membrane integrity maintenance and the enzymes and proteins stability[14,15]. Salinity triggers variations in the cellular and molecular levels and hence induces some specific genes expression[16].

The salinity stress related genes are a wide array of diverse type of genes. These genes are from energy metabolism, ion trans-membranes, photosynthesis, signal transduction and some other metabolic pathways. Some genes are salinity specific. Therefore, salinity stress may induce variations in the gene expression equilibrium going to the adaptation to the specific stressful environments[17,18]. Previous studies have revealed that, salinity depression imposes huge gene expression pattern variations in grapevine[19].

In vitro culture is an efficient alternative means to simulate the complex interactions of the environments and soil with the physiology of the plants[8,20]. Furthermore, under *in vitro* conditions and especially during the screening stages in breeding programs; the environmental restrictions would be resolved easier than common soil based studies[21]. *In vitro* screening for the salt tolerance in grapevine[8,22] is an acceptable recommended procedure and there are some techniques for the efficient screening in mulberry[23] citrus trees[24] and cherry rootstocks[25]. The present study was planned to assay the *in vitro* response of some grapevine cultivars to the salinity stress by the tracing of morphological and enzymatic responses as well as the defined genes expression pattern variations.

Material And Methods

Plant material, experiments and treatments.

Two grapevine cultivars (Fakhri and Sultanin) were imposed to salinity treatments of 0.25, 0.5, 0.75 and 1 $gL^{-1}$of NaCl. Single nodal explants were taken from the mother plants, disinfected with 70% ethanol for 1 min, followed by sodium hypochlorite 20% for 20 min. Then, the explants were three times rinsed with distilled sterile water. The Murashige and Skoog (MS) medium containing NaCl treatments were supplemented with vitamins and 1.5 mg/l BAP and 1 mg/l IBA plus 30 g/l of sucrose. pH was adjusted to 5.8 and the media were autoclaved at 121°C. Single node cutting of 1 cm length were the materials taken for culture initiation.

To assess the antioxidant enzymes, MDA, protein content, and gene expression pattern; plant samples were incubated at -80$^{\circ}C$. Other traits were measured after the first round of culture (2 months after the initial explant inoculation). The parameters included: stem length, fresh weight (mg), viability (%), shoots number, proliferation ratio, leaves number per shoots and the number of shoots with three nodes.

Antioxidant enzymes assay.

To have the extract of CAT, GPX, SOD and soluble proteins; 0.2 g of plant material (shoots after 2 month inoculation) was digested in liquid nitrogen. 2 ml of phosphate buffer (pH= 7.5) containing EDTA (0.5 mol) was added. Then after, the extracts were centrifuged at -4$^{°C}$ for 15 min at 15000 rpm, then collected the supernatant and stored it for assay[26].

Due to the short half-life of APX under ex-vitro condition and for keeping the cell structure and components; PVP (5%) and 2 ml ascorbate was added to the enzymatic solution. APX was assayed as; the reaction mixture was containing 250 µL phosphate buffer (pH=7) along with EDTA, 10 µL H_2O_2 (1 mmol), 250 µL sodium ascorbate (0.25 mmol) and 50 µL enzyme solution. The absorbance was measured at 290 nm[27].

For the activity measurement of GPX the reaction mixture was contained 1 ml phosphate buffer (100 mmol, pH=7) along with EDTA (0.1 mmol), 1 mL guaiacol (15 mmol), 1 ml H_2O_2 (3 mmol) and 50 µL of the extracted enzyme solution. The reaction response was measured at 470 nm for 1 min[28]. For CAT, the reaction mixture was containing 1.5 ml phosphate buffer (100 mmol, pH=7), 0.5 ml H_2O_2 (7.5 mmol) and 50 µL of extracted enzyme solution. The absorbance at 240 nm during 1 minute was measured[29]. SOD activity was evaluated in a reaction mixture having 1.6 ml phosphate buffer (100 mmol) containing 0.1 ml EDTA, and sodium carbonate (1.5 M), 0.2 ml of L-methionine (0.2 M), 1 ml of distilled water, 0.1 ml of NBT (2.25 mM) and 50 µL of enzyme solution. The enzyme reaction and activity was initiated with the addition of 0.1 ml riboflavin (60 µL) and the samples absorbance was recorded at 560 nm[30].

Malondialdehyde assay.

0.2 g of plant sample was homogenized in 2 ml of TCA (20%) containing 0.05% TBA. The samples were incubated at 95$^{°C}$ for 30 minutes and then were transferred on ice. The samples were centrifuged at 10000 rpm for ten minute and the absorbance was recorded at 532 and 600 nm[27].

Soluble protein assay.

To prepare the solution, 100 µl of enzymatic solution, 200 µl of Bradford reagent and 700 µl of deionized water were mixed. 2 min after the complex formation of Bradford reagent (interaction with the amino acids), the absorbance was recorded at 523 nm. Proteins content of the samples was calculated based on cow albumin-serum calibration curve[31].

Data were analyzed by SPSS (v. 21) and mean comparisons were accomplished by Duncan's multiple range test.

RNA extraction and DNA synthesis.

Total RNA was extracted from the *in vitro* derived shoots and the RNA extract was purified by the method of Tatterall et al.[32].

The quantification and qualification of the extracted RNAs was carried out by the A260/A280 ratio (1.8-2) and A260/A230. The extracted RNAs were identified in 2% agarose gel by the golden red staining method. The reaction mixtures (Table 1) were prepared in microtubes on ice and reached 20 µl by RNase-free water.

Primer design and RT-qPCR analysis.

RNA sequences of genes of *VvCBF*$_{1,2,3,4}$ and *MDH* (Reference gene) were acquired from NCBI (www.ncbinlm.nih.gov) and the forward/reverse primers were designed by oligo 7 software (Table 2). RT-qPCR analysis was done by ABI StepOne (Applied biosystems) and were detected by SYBR Green PCR Master Mix (TakaRa, Toyo to, Japan). Reaction mixture (Table 3) of 20 µl was prepared for each sample.

Initially, denaturation was done at 5$^{°C}$ for 5 min. 40 cycles of 95$^{°C}$ for 5 sec and 60$^{°C}$ for one minute. After calculation of the amplification efficiency of the primers; the reaction was done by the method of $2^{-\Delta\Delta CT}$. The analysis of Real-time PCR data was accomplished based on the threshold cycle acquired for the target and reference genes. The difference between ct reference genes from the mean of ct target gene was considered as Δct index for both the test and control groups. ct_s difference of test and control groups were employed for the calculation of ΔΔct.

Results

Morphological traits.

The results for the explants exposed to the salinity during 2 months with one sub-culture showed that the viability statistically declined with salinity intensity especially in 'Sultanin' cultivar. In 'Fakhri', 0.25 and 0.5 gL^{-1} of salinity had no meaningful effect on the viability of explants. Shoot growth in both cultivars correspondingly declined in response to the salinity. The fresh weight of the regenerated explants was impacted by the salinity compared to the control. So that, at 1 gL^{-1} salinity, the fresh weight was statistically lower than control and other salinity levels. Shoot number was not affected by the salinity treatment at any levels (Table 4).

Proliferation rate was declined with the salinity; however, there was no noticeable difference between the salinity levels. Leaf number per shoot in'Fakhri' was not influenced by the salinity of up to 0.75 gL^{-1}. But, in 'Sultanin', there were significant differences between the control and saline treatments considering the number of leaves. The number of three nodal shoots per explant was drastically impacted by 1 gL^{-1} salinity in both cultivars.

Biochemical parameters.

For the APX activity and for both cultivars, there were no differences between control and NaCl treatments. But the APX activity of cultivar 'Fakhri' was significantly higher than that of the other cultivar. GPX attained the top-activity with 'Fakhri' (Fig. 1).

The highest recorded activity of CAT was at 0.5% and 1 gL^{-1} for 'Fakhri' and 0.25 gL^{-1} NaCl for 'Sultanin'. The least data for CAT was belonged to control plants of cultivar 'Sultanin'. SOD activity in 'Fakhri' was correspondingly increased with salinity from 0.25, 0.75 gL^{-1}. However, in 'Sultanin', there was no significant difference in SOD activity between control and the saline treatments up to 0.75 gL^{-1}. Significant SOD activity differences were observed between the cultivars (Fig. 2).

MDA content as the biomarker of membrane lipids peroxidation was uniformly responded to the salinity levels in both cultivars. Soluble proteins content in both cultivars was diminished. However, the reduction was more highlighted in 'Fakhri' (Fig. 3).

Correlation coefficient.

As shown in table 5, the positive or negative response were observed between the traits in response to salinity stress. Considering the significant positive relationship was recorded between the shoot lengths with the fresh weight of proliferated explants, variability, shoot number, proliferation rate and leaves number. Furthermore, positive and significant correlation was calculated in between the APX activity and the activity of GPX and CAT. Moreover, the relationship was observed between GPX with CAT and SOD. Alongside, negative correlations were recorded between the activity of SOD and GPX with MDA. Overall, considering all the results, the traits Y1 to Y7 and the activity of enzymes; APX, GPX and SOD would be reliable criteria in assessing the grapevine genotypes against the salinity stress and to select the promising salt tolerant grapevine cultivars.

Factor and bi-plot analysis.

Using the first three main factors, the proportional effect of the traits on the main factors were depicted by a bi-plot chart (Fig. 4). The results of the bi-plot analysis revealed the traits; stem length, the fresh weight of proliferated explants, viability, shoot number, proliferation rate and leaves number had reasonable relationships. Furthermore, factor analysis showed that the two major factor with the top proportional rate 60.395% of total variations were explained by the variables. The first main factors with the highest positive impact on the observed variation were Y1 to Y7. For the second main factor, the traits Y8-Y11 were the most influential positive ones. But, Y12 had the most negative and bi-plot analysis is in agreement with correlation results that Y1-Y7 significantly correlated to each other (Table 6 and 7).

Gene expression.

Expression pattern of *DREB/CBFs* genes in response to the *in vitro* salinity was also concentration dependent and, showed an ascending pattern with increase in salinity. Expression of $CBF_{2,3}$ and $_4$ were increased with salinity of 0.25 to 1 gL^{-1} and compared to control in both cultivars. *CBFs* expression in 'Fakhri' was more than 'Sultanin' in response to the salinity. For CBF_4, with salinity up to 1 gL^{-1}; the expression was 5.66 and 4.25 fold more than control in 'Fakhri' and 'Sultanin', respectively.

CBF_3 expression was 3.95 and 2.94 fold higher than control for 'Fakhri' and 'Sultanin' again with the highest salinity level tested. At 0.25 gL^{-1} salinity level, *CBF3* expression did not show significant differences between the treatments. The same trend of expression pattern was followed for CBF_2 with up to 1 gL^{-1} salinity (2.45 and 2.14 fold increase for 'Fakhri' and 'Sultanin', respectively). However, there were no differences between cultivars with regards to salinity levels (Fig. 5). The most recorded CBF_1 expression for both cultivars was belonged to 0.75 gL^{-1} salinity level. Meanwhile, the trend was decreasing with the higher level of salinity (1 gL^{-1}).

Discussion

The salinity effects on the growth characteristics were evident. Salinity stand-up in the growing media restricted the growth potential of explants with regard to the proliferation rate, fresh weight, viability, shoot length and three nodal shoots number.

The *in vitro* and *in vivo* effects of salinity treatments on 'Sultanin'[7,33,34] Black Corinth and Emperor[35] Perlette, Beauty seedless and Delight[36] grapevines have been previously reported.

The previous data shows that the salinity treatments up to 1 gL^{-1} in 'Sultanin' grape drastically reduced the explants viability[7]. High Na^+ concentrations in the growing media antagonistically prevent the absorption of other cations. Researchers demonstrated that the more sodium availability limited Mg^{2+}, Ca^{2+} and K^+ intake in grapevines. Salinity restrains the overall growth and development of plants[2]. The others reported the same idea emphasizing that the shoots growth in grapevine was greatly inhibited by the action of Cl^- ions[3,37].

Salinity damage is dependent upon the NaCl concentration, treatment time-course, and cultivar type. Several researchers have verified the mentioned side effects of salinity on plant[35,38]. Salinity impedes the vegetative and reproductive growth by the direct or indirect action on the physiological dynamics of cells and whole plants. The damages on tissues come from not only the osmotic pressure but also, more intensified by the toxic effects of Na^+ and Cl^- ions[2,3].

In the present study, APX enzyme activity in 'Fakhri' under the diverse salinity levels had no significant differences with the control ones. However, in 'Sultanin' grape, the APX activity was increased with salinity. In another study on apple and pear cultivars in MS medium, the highest APX activity was devoted to 150 µm of NaCl[25,39].

For GPX, the highest data were recorded with 0.5 gL^{-1} of NaCl. The increased levels of GPX have been frequently reported in salt tolerant species. Some documents say that this enzyme activity has been increased in both sensitive and tolerant cultivars. However, the increasing pattern and rate are different in diverse species[40,41].

CAT activity was increased in 'Sultanin' grape in line with salinity levels. On the contrary, in 'Fakhri', there was no significant difference in CAT activity between shoots under various salinity treatments. Sweet cherry rootstocks showed broad diversity in CAT activity *in vitro* exposed to salinity treatments[25]. The similar results are available for apple[39]. So, all the antioxidant enzymes do not follow a liner increase in the activity in response to the salinity. Since, the salinity stress treated feedback of plants is dependent on species, growth stage, salts concentration and type, treatment exposure times and absolutely, the activity would be different for the diverse enzymes[11,42].

SOD activity was initially increased for all the salinity treatments; but, later declined. Under the high salinity concentrations, the biochemical responses of plants are not correspondingly related to the salinity treatments, although the growth and yield attributes are negatively affected. With increasing the salinity levels, the antioxidant system of plants is activated and hence with the production and activity of SOD as the forefront barrier against ROS radicals; the tissues and whole body of the plants get ready to face with the salinity stress[39]. Furthermore, with high salinity levels and the age of the plants, the SOD activity was considerably increased[43]. The elevated SOD activities under the salinity stress have been reported for mulberry[44], citrus crops[45] and maize[46].

Salinity stress triggers oxidative membranes deterioration. Plants evolved some mechanisms to denature and decline the activity of ROS molecules by motivating the antioxidants potential to control the stress damages. The first step in this passage is the scavenging of H_2O_2 to protect the damages caused at cellular, and tissue level and to mitigate the oxidative stress[47,48].

With salinity, MDA levels were increased. Lipids peroxidation coincides with the increased antioxidant enzymes activity in the salt tolerant plants. MDA ameliorates the oxidative stress and intensifies the scavenging of ROS radicals[46,49].

Many findings have noted that the consequence of ROS molecules over-production is the membrane lipids peroxidation leading to the production of aldehydes and specially MDA and some other products like ethylene[50]. The production of these compounds can be annotated as the lipids peroxidation and fatty acids oxidation and their structural denaturation. The high MDA content under salinity conditions is an outcome of the reduced content and activity of SOD, GR and CAT[39,44,48]. Salinity apart from the lipids deterioration denatures membrane-anchored proteins and hence reduces the total protein content of plants. ROS radicals' over-activity under the salinity environments oxidase the amino acid chains and finally denatures proteins and alters their conformational moiety[46].

The same trend was followed for CBF_4 and the expression rate in cultivar 'Fakhri' was higher than that in cultivar 'Sultanin'. CBF_1, and in 'Fakhri', the expression was additive up to 0.75 gL^{-1} of NaCl. But, then after decreased. In 'Sultanin', the CBF_1 expression rate with salinity of up to 0.75 gL^{-1} declined but, was higher again at 1 gL^{-1}.

CBF/DREB proteins are fundamental compounds in the expression of specific genes involved or responsive in abiotic stresses tolerance. The mentioned proteins are target compounds of the breeding programs for the environmental stressors. The majority of *CBF* genes are characterized to be influential in the induction of tolerance responses to the low temperature stress[51,52,53], Otherwise, several studies have claimed that *DREB/CBF* genes are induced by the several stressors and, ABA activates a bunch of *DREB/CBF* genes[51,54,55]. CBF_4 is the major gene respondent to the drought stress. CBF_4 expression is more activated by the exogenous application of ABA in Arabidopsis and grapevine. CBF_{1-3} expression was also enhanced with the ABA external applications in main part due to the increased activity of *CBF* promoters in reply to ABA[54,56,57]. The differences in *CBF* proteins in grapevine reflect the positioning of CBF_4 in the separate phylogenetic groups compared to $CBF_{1,2,3}$[19,58,59]. This possibly justifies that CBF_4 in grapevine behaves in different manner than $CBF_{1,2}$ and 3. $CBF_{1,2,3}$ are more expressed in young tissues[60]. However, CBF_4 is typically under the active expression in the young and mature leaves and in the nodes. Seemingly, *CBF/DREB* genes act as connector or interaction point of several biosynthetic pathways and simultaneously regulate the tolerance to the stressors like drought, cold and salinity. Transgenic Arabidopsis lines of *DREB1B/CBF1* or *DREB1A/CBF3* exhibited reasonable salinity tolerance. It seems that *DREBs/CBFs* target several genes[61].

CBFs genes play inimitable role in the growth and development of grapevine plants. So, may be employed as biomarkers in the screening of salt tolerant cultivars in the breeding programs of grapevines.

Conclusions

The growth parameters were evaluated in two-grapevine cultivars *in vitro* with four salinity treatments. Shoot length, explant weight, viability, number of shoots, proliferation ratio and the number of 3 nodal shoots were significantly decreased by salinity. The explants viability in 'Sultanin' significantly decreased with increasing salinity levels. The growth parameters in 'Sultanin' showed significant decrease compared to 'Fakhri' under different levels of salinity. However, there was no significant difference response of explants to 0.75 and 1 g/l of salinity except for the viability trait. The activity of antioxidant enzymes APX, GPX, CAT and SOD in 'Fakhri' cultivar was higher than that of 'Sultanin' with the diverse salinity levels. 1 g/l salinity significantly reduced the activity of SOD. As salinity levels increased, malondialdehyde content was increased in both cultivars. The expression rate of $CBF_{2,3,4}$ genes showed a significant raise with salinity. CBF_1 expression rate was increased with the salinity of up to 0.75 g/l in both cultivars. 'Fakhri' cultivar was more tolerant to the salinity more possibly due to its high antioxidant capacity and the high expression of *CBFs* genes.

Declarations

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on request.

Author contributions

Study conception and design, performed experiments, drafting of manuscript done by Aazami; analysis of data, improvement of the manuscript done by Hassanpouraghdam.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Additional information

Correspondence and requests for materials should be addressed to M.A. Aazami.

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Tables

Table 1. Reaction mixture for cDNA synthesis

Volume	Reactive
0.5 µl	Vivantis RT Enzyme Mix
2 µl	Buffer RT Enzyme
0.5 µl	Oligo RT Primer (50 µM)
0.5 µl	Random 6 mers (100 µM)
1 µl	dNTP
11.5 µl	DDW
5 µl	Total RNA (500 ng)
20 µl	Total

Due to technical limitations, table 2 is only available as a download in the Supplemental Files section.

Table 3. The composition of reaction mixture for RT-RCR

Volume	Reactive
2 µl	RT reaction solution (cDNA)
0.4 µl	Primer F
0.4 µl	Primer R
10 µl	Power SYBR Green PCR Master Mix
7.2 µl	DDW
20 µl	Total

Table 4. The effects of NaCl treatments on some physiological traits in two grape cultivars

Cultivar	NaCl(gL^{-1})	Shoot length (mm)	Shoot weight (mg)	Viability (%)	Number of shoots	Proliferation ratio	Number of leaves/shoots	Number of 3-node shoots
	0	35.13±0.99a	324.79±41.66a	100±0a	6±0.61a	14.25±3.36a	4.5±0.25a	2.75±0.21a
	0.25	23.62±0.77b	267.04±17.87ab	87.50±2.16b	5±0.35a	8.75±0.41bc	3±0.35bcde	2.25±0.21ab
Sultanin	0.5	16.33±0.81c	182.12±31.72bc	86.25±3.75b	4.25±0.89ab	5.5±0.55cde	2.75±0.21cdef	1.75±0.41abcd
	0.75	10.18±0.45d	93.05±6.82cd	76.25±2.72c	2.25±0.41bc	3±0.61de	2.25±0.21ef	1.5±0.25abcd
	1	7.93±0.34d	27.59±4.64d	21.25±3.69e	1.5±0.43c	1.75±0.41e	1.75±0.41f	0.5±0.25d
	0	33.38±3.25a	314.72±16.14a	100±0a	6.25±0.96a	11.75±2.21ab	4±0.35ab	2.75±0.21a
	0.25	22.08±0.59b	294.67±70.3ab	100±0a	4.5±0.55ab	7.5±0.55bcd	3.75±0.21abc	2.75±0.21a
Fakhri	0.5	16.49±0.71c	263.74±37.74ab	100±0a	4.25±0.96ab	5.25±0.21cde	3.5±0.25abcd	2±0.5abc
	0.75	11.96±0.52d	105.86±11.09cd	83.75±3.69bc	2.75±0.41bc	2.75±0.21de	2.75±0.41cdef	1.25±0.54bcd
	1	9.10±0.64d	49.92±11.01d	33.75±2.72d	2.25±0.21bc	1.25±0.21e	2.5±0.25def	0.75±0.41cd

Means by the same latter within each column are not significantly different at 0.05 level, according to the Duncan's multiple range test. Data are mean±SD (n=4 replicates)

Table 5. Coefficients of correlation values for the pairs of studied characters in two grape cultivars under NaCl

	Y1 (Shoot length)	Y2 (Shoot weight)	Y3 (Viability)	Y4 (Number of shoots)	Y5 (Proliferation ratio)	Y6 (Number of leaves/shoots)	Y7 (Number of 3-node shoots)	Y8 (APX)	Y9 (GPX)	Y10 (CAT)	Y11 (SOD)	Y12 (MDA)	Y13 (Pro
Y1	1												
Y2	0.769**	1											
Y3	0.670**	0.734**	1										
Y4	0.766**	0.742**	0.626**	1									
Y5	0.841**	0.728**	0.575**	0.692**	1								
Y6	0.711**	0.689**	0.617**	0.544**	0.591**	1							
Y7	0.718**	0.760**	0.633**	0.597**	0.545**	0.652**	1						
Y8	0.033	-0.047	-0.207	-0.016	0.090	-0.025	-0.071	1					
Y9	0.102	-0.013	-0.041	-0.035	0.178	-0.057	0.043	0.466**	1				
Y10	-0.053	-0.135	-0.114	-0.058	0.040	-0.126	0.017	0.679**	0.399*	1			
Y11	0.076	0.098	0.126	-0.028	0.215	-0.022	0.197	0.302	0.507**	0.261	1		
Y12	0.134	0.074	-0.106	0.272	0.038	0.121	0.188	-0.272	-0.377*	-0.253	-0.328*	1	
Y13	0.376*	0.098	0.065	0.130	0.377*	0.128	0.132	0.230	0.025	0.116	0.278	-0.046	1

**, * significant at p≤0.01 probability and significant at p≤0.05 probability, respectively.

Table 6. Total variance explained			
	Initial Eigenvalues		
Components	Total	% of Variance	Cumulative %
1	5.172	39.783	39.783
2	2.680	20.612	60.395
3	1.132	8.710	69.105
4	0.991	7.620	76.725
5	0.739	5.688	82.412
6	0.587	4.512	86.925
7	0.483	3.716	90.641
8	0.358	2.753	93.394
9	0.273	2.102	95.496
10	0.243	1.872	97.368
11	0.156	1.201	98.570
12	0.123	0.946	99.516
13	0.063	0.484	100.000
Extraction Method: Principal Component Analysis.			

Table 7. Total variance explained		
Component Matrix[a]		
	1	2
Y1	0.930	0.060
Y2	0.905	-0.053
Y3	0.847	0.198
Y4	0.832	-0.097
Y5	0.823	0.002
Y6	0.802	-0.070
Y7	0.797	-0.099
Y8	-0.040	0.788
Y9	0.032	0.758
Y10	-0.083	0.733
Y11	0.138	0.672
Y12	0.139	-0.560
Y13	0.274	0.334
Extraction Method: Principal Component Analysis (PCA). 1		
(PCA1) and 2 (PCA2) a: 3 components extracted. Y1 (Shoot length),Y2 (Shoot weight),Y3 (Viability),Y4 (Number of shoots),Y5 (Proliferation ratio),Y6 (Number of leaves/shoots),Y7 (Number of 3-node shoots),Y8 (APX),Y9 (GPX),Y10 (CAT), Y11 (SOD),Y12 (MDA),Y13 (Protein)		

Figures

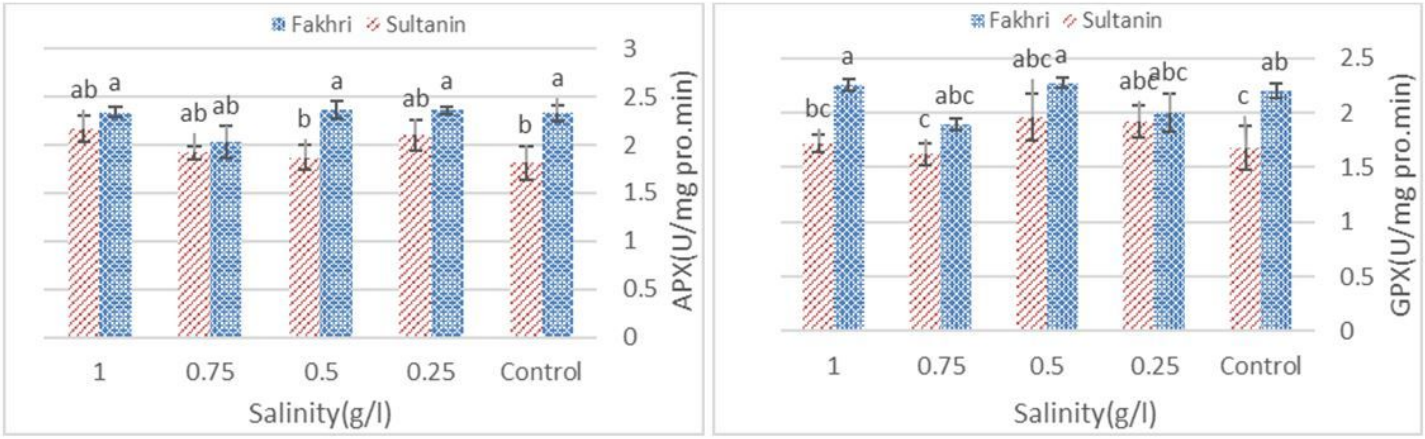

Figure 1

The effects of NaCl treatment on APX and GPX activity of two grapevine cultivars; 'Fakhri' and 'Sultanin' in vitro. Means followed by the same letter on columns are not significantly different at 0.05 level, according to the Duncan's multiple range test. Data are mean±SD (n=4 replicates).

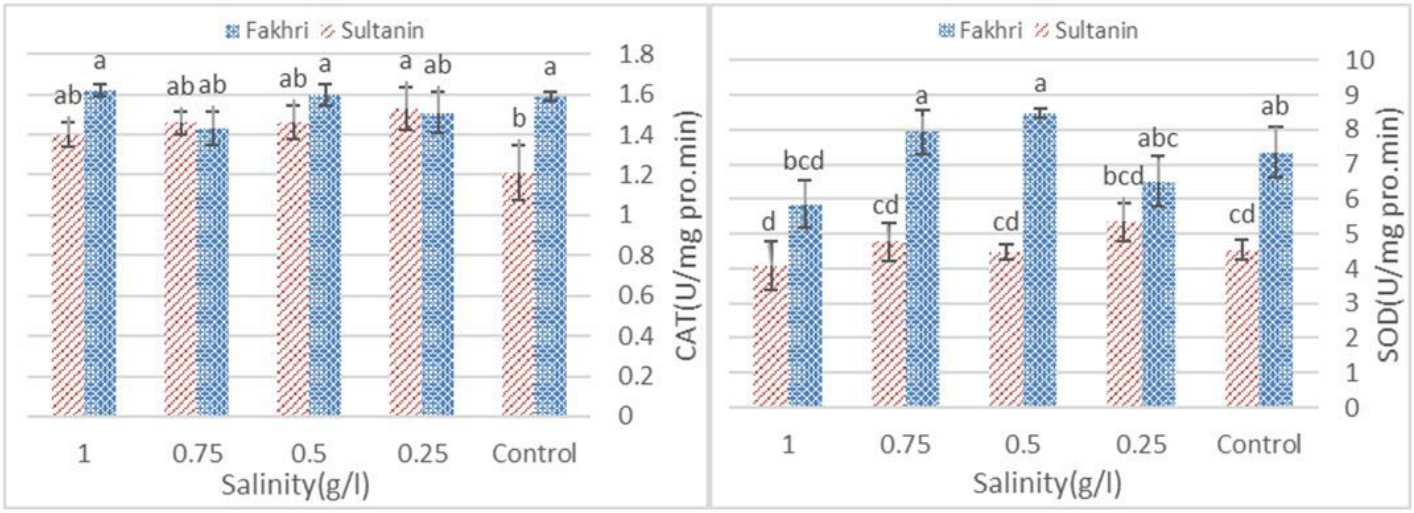

Figure 2

The effects of NaCl treatment on CAT and SOD activity of two grapevine cultivars; 'Fakhri' and 'Sultanin', in vitro. Means followed by the same letter on columns are not significantly different at 0.05 level, according to the Duncan's multiple range test. Data are mean±SD (n=4 replicates).

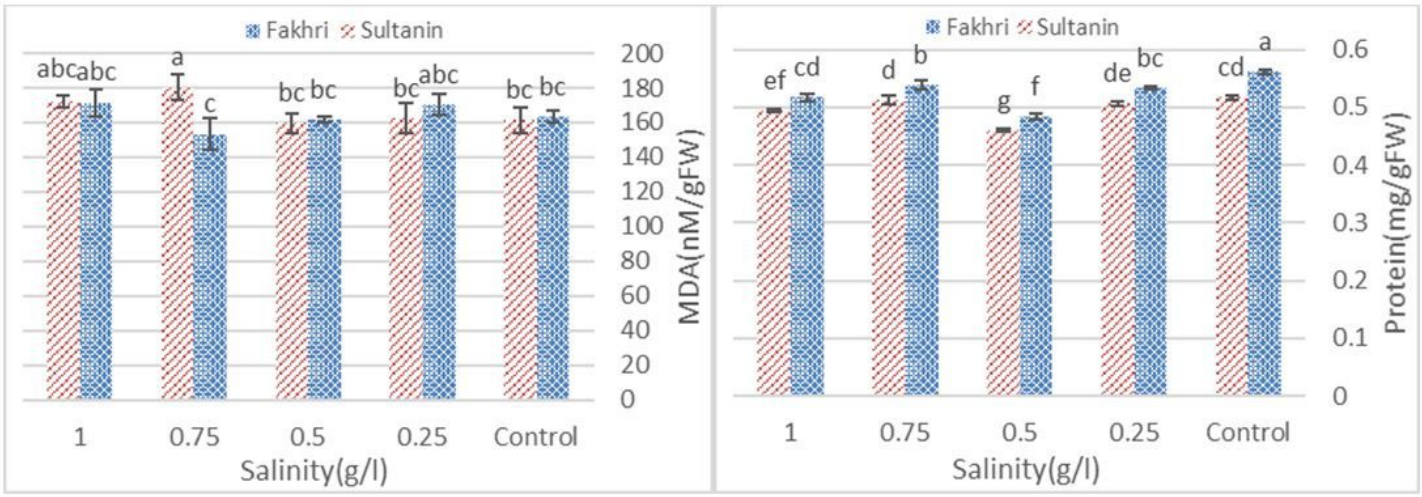

Figure 3

The effects of NaCl treatment on MDA and protein content of two grapevine cultivars; 'Fakhri' and 'Sultanin', in vitro. Means followed by the same letter on columns are not significantly different at 0.05 level, according to the Duncan's multiple range test. Data are mean±SD (n=4 replicates).

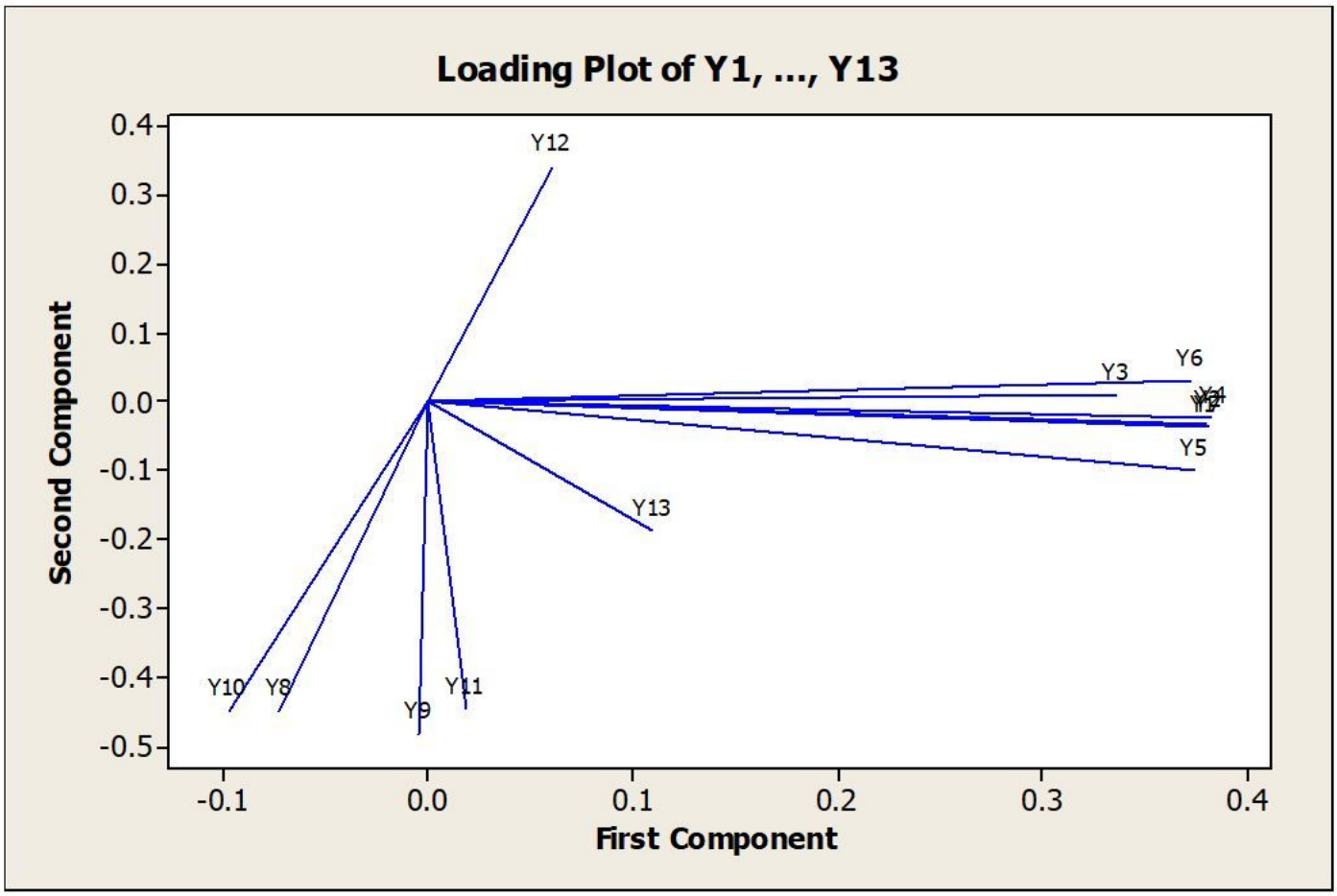

Figure 4

Bi-plot demonstration of 13 traits of 2 grape cultivars based upon principal components. Y1 (Shoot length), Y2 (Shoot weight), Y3 (Viability), Y4 (Number of shoots), Y5 (Proliferation ratio), Y6 (Number of leaves/shoots), Y7 (Number of 3-node shoots), Y8 (APX), Y9 (GPX), Y10 (CAT), Y11 (SOD), Y12 (MDA), Y13 (Protein).

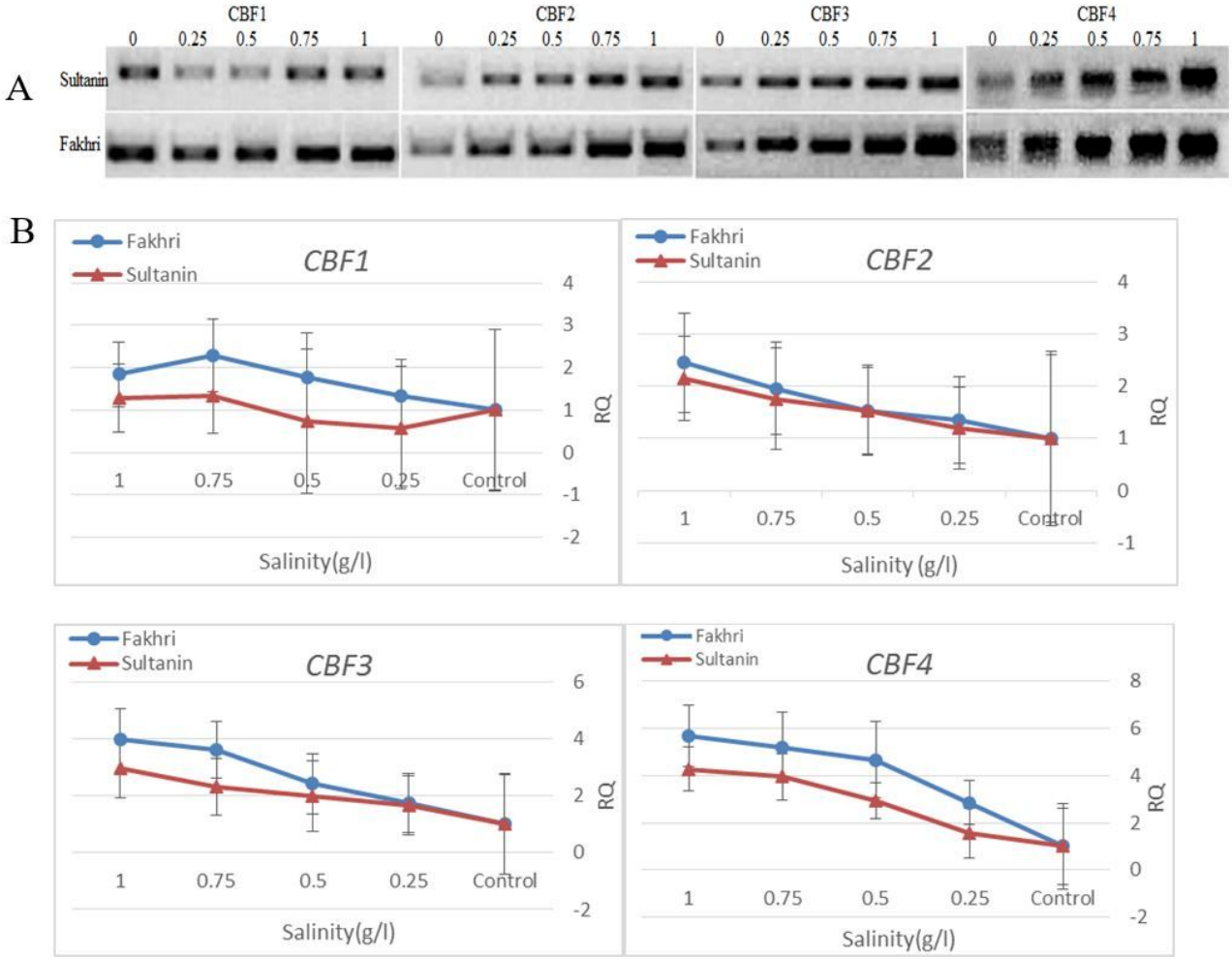

Figure 5

Semi-quantitative (A), Quantitative (B) expression analysis of DREB/CBFs genes in two grapevine cultivars exposed to salinity in vitro condition. Data are mean±SD (n=3 replicates).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2.jpg