Evaluation of traits and protein changes of Fornax cultivar of canola under salt stress treatment

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Abstract

Canola oil is a vegetable oil derived from a variety of rapeseed with lower erucic acid contents. To investigate the mechanism of response to salinity induced by sodium chloride in canola, Fornax cultivar was evaluated in greenhouse conditions. Results showed that salinity stress affected morphological and physiological traits so that leaf relative water content and photosynthesis-related traits decreased while proline and glycine betaine concentrations were higher than in control plants. In addition, under stress conditions, imbalances in ionic concentrations were caused by an increase in sodium ion concentration and a decrease in intracellular potassium ion, as well as a decrease in the potassium / sodium ratio in leaf cells. Proteomic analysis results also detected 7 protein spots with expression difference and significant induction factor (IF) between control plants and severe salinity stress. Each protein, identified by mass spectrometry, was related to antioxidant defense, involved in photosynthetic light reaction, Calvin cycle, and nitrogen assimilation, respectively.

Keywords: oil crop, greenhouse, proteomic analysis, salinity

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Introduction

Salinity stress is one of the most important abiotic stresses and its negative effects on crop growth have motivated research to improve plant tolerance (Jin et al., 2021). Not all plant varieties have the same response to salinity stress, and genotypes with higher tolerance to salinity are likely to exist (Munns and Tester, 2008). Salinity tolerance is a quantitative trait that is controlled by multiple genes. Some of these genes have been identified and can be used in plant breeding and screening programs (Ashraf and Mc Neilly, 2004). Salinity tolerance is a complex process, because salinity stress causes loss of tissue water, increased ion toxicity, nutritional imbalance, or a combination of these (Pandey and Penna, 2017).

In addition to decreasing plant height, salinity stress decreases leaf area and total dry matter and consequently, decreases crop yield. Due to salinity stress, leaf area and plant height decrease much faster than other morphological indices and tolerant genotypes tend to maintain high amounts of these variables (Munns et al., 2006). Therefore, it is recommended to base selection for salinity tolerance on physiological, morphological, and biochemical characteristics. In this regard, the use of biochemical markers such as proline accumulation, glycine betaine, sugars or ratios, etc. have been more widely used (Pandey and

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Penna, 2017). In addition to osmotic and ionic toxicity, soil salinity causes nutritional imbalances in the plant, the severity and weakness of which depend on the plant type and even the different species of a plant (Munns et al., 2006). In plants under stress, K^+ is transferred to the leaves while Na^+ is distributed more evenly. Hence, K^+/Na^+ ratio in plants under normal conditions is higher than those under stress. This ratio decreases with increasing environmental salinity (Pandey and Penna, 2017). Lower Na^+ accumulates in plant tissue and, as a consequence of K^+ increase in tissue, leads to increased K^+/Na^+ ratio in plants thereby, increasing plant tolerance to salt (Houshmand et al., 2005). Also, in saline conditions, plants need special mechanisms to regulate internal osmotic conditions so as to reduce their osmotic potential and thus reduce osmosis and also pressure in root environment through free amino acids, soluble sugars, and proteins (Mahmood and Athar, 2003). salinity increases accumulation of magnesium (Valdez-Aguilar et al., 2009), total starch content (Pessarakli et al., 2005), and accumulation of simple sugars such as glucose and fructose. This is due to increased invertase enzyme in leaves of the plants under stress (Pinheiro et al., 2001).

In canola seedlings, the relative water content was found to decrease gradually with increasing electrical conductivity (Francois, 2007; Qi-lin et al., 2009). Proline is an osmotic regulating osmolyte and its distribution stabilizes the cell wall structure and proteins and cleans free radicals. The rate of proline accumulation under moderate or severe stress increases compared to other amino acids (Saadia et al., 2012).

Glycine betaine is also an important adaptation solution that accumulates in response to osmotic stress (Pandey and Penna, 2017). On the other hand, photosynthetic capacity of chloroplasts decreases due to salinity stress as salinity stress disrupts chloroplast structure, destabilizes pigment protein complexes, degrades chlorophyll structure, and changes carotenoid contents and their composition (Kattab, 2007). Chlorophyll level of leaves is a useful index for assaying photosynthetic potential and general power of the plant (Alonso et al., 2002). Chlorophyll fluorescence measurement is also a relatively new technique used to evaluate plant photosynthetic activities (Kocheva et al., 2004). This technique is based on physiological calculations and measures the efficiency of light harvesting and absorption mechanisms in relation to the activities of chlorophyll photosystem II (Maxwell and Johnson, 2000). The Fv/Fm ratio has been used as a reliable selection index for stress conditions, and it has been reported that the rate of photochemical performance in photosystem II or Fv/Fm reduces under stress conditions (Schreiber et al., 1994). Overall, salt tolerance in plants is a complex phenomenon and is associated with a variety of physiological, biochemical, and molecular mechanisms; therefore, understanding the molecular mechanism of stress response along with other studies is important.

Abiotic stresses usually cause protein disorders. Plants overcome stress induced by the synthesis of some essential metabolites in the face of stress conditions. This is done by altering the expression of genes to reduce or increase the amount of specific structural or enzymatic proteins involved in metabolic pathways. Not only can these proteins be used as markers, they also play an essential role in adapting to environmental stresses (Joseph and Jini, 2010). Therefore, studying these proteins to identify molecular mechanisms of the plants' tolerance to abiotic stresses seems necessary.

Proteomic approach is a powerful and appropriate tool for investigating expression changes and identifying proteins that respond to environmental stresses (Sobhanian et al., 2011). In fact, proteomic analysis has been reported as one of the best methods to evaluate protein expression dynamically under salinity stress (Komatsu and Tanaka, 2004).

Photosynthesis is one of the most important processes in plants in which the identification of altered proteins expressed under stress is very important. For example, the oxygen evolving photosystem II protein is one of the proteins involved in photosynthesis. Oxygen evolving complex (OEC) is part of these proteins and plays a role in stability of photosystem 2 (PSII). In addition, this protein also plays a role in manganese stability through its association with

the Manganese complex of OEC. In other words, the activity of this protein is the production of oxygen in the electron transfer cycle (Holmstrom et al., 2000). Furthermore, $PSBO^{-2}$ proteins, which have been implicated in studies of the effect of salinity stress on protein alteration patterns, belong to the oxygen evolving family and maintain the Mn group, which is the major site of water molecule breakage (Kumara et al., 2003). On the other hand, the most important enzyme active in the Calvin cycle is Ribulose-1,5-bisphosphate carboxylase/oxygenase, which has two functions and is involved in the early stages of two opposing metabolic pathways, namely photosynthetic carbon stabilization (in the Calvin cycle) and the optical breathing process. Although large subunits carry the activity of this enzyme, it has been shown that small subunits increase their activity by modifying the structure of large subunits (Vaidyanathan et al., 2003). Yıldız et al. (2015) in the study of canola leaf proteome under salinity stress have pointed to increased expression of this enzyme and its correlation with a mechanism that enhances plant photosynthetic power. It should be noted that as stress conditions increase, the frequency of hydroxyl radicals causes this to break down the large subunit.

In salt stress conditions, plants are also subjected to oxidative stress. One of the major pathways for ROS scavenging in plants is the activity of antioxidant enzymes such as superoxide dismutase (SOD) proteins that are involved in the conversion of superoxide radicals to molecular oxygen (Cakmak, 2005). On the other hand, peroxiredoxin proteins are widely expressed in tissues and are found in mitochondria, peroxisomes and cytosol. The cellular location of these proteins indicates that they play an important antioxidative role in the cellular organelles that are the major source of ROS. In addition to antioxidant activity, these proteins are also active in controlling signal transduction. It can be argued that copper/zinc superoxide dismutase activity converts ROS to H_2O_2, and H_2O_2 is reduced particularly by type 2 peroxiredoxin enzyme (Hashimoto, et al., 2009).

The purpose of this study was to investigate the morphological, physiological, and biochemical traits of Fornax cultivar of canola and the two-dimensional protein electrophoresis pattern of this sensitive cultivar under salinity stress, identifying proteins with altered expression by mass spectrometry and finally, the role of these proteins in metabolic pathways of the cultivar.

Materials and Methods

Seeds of Fornax salinity-sensitive canola were prepared from Karaj Seed and Plant Improvement Institute, Iran. The experiment was conducted as a randomized complete block design (RCBD) with three replications in a research greenhouse by hydroponic cultivation in 2018. NaCl salinity was applied at three levels of zero (control), 150, and 300 mM. Seedlings were planted in the main experimental environment after vernalization. For this purpose, the seedlings were transferred to the growth chamber in 5-6-leaf stage and were stored at 2-4 ℃ for 10 hours per day for 8 weeks. After this period, the seedlings were transferred to the greenhouse so that the plants started to grow. Then, the salinity stress was applied and gradually increased to 50 mM/day until reaching the desired level. Leaf sampling was performed and the studied traits were measured using appropriate methods two weeks after full stress on the seedlings. Relative water content of leaves was determined by Morant-Manceau et al., (2004). To determine leaf water potential and osmotic potential, a pressure chamber (Soil Moisture Equipment Crop, Sanat Barbara, CA) and an osmometer (Osmomat o10, Gonotec) were used, respectively. Also, chlorophyll fluorescence, chlorophyll index, and Na^+ and K^+ ions were assayed using a fluorimeter (Opti Science, OS-3OMSA model), a chlorophyll meter (SPAD-502, Mlolta, Japan), and a flame photometer (Genway Model PFP7/C), respectively. Also, leaf proline concentration and glycine betaine contents were determined by ninhydrin method (Bates et al., 1973) and Grieve and Grattan method (1983) with a spectrophotometer, respectively. Plant height and dry weight were also measured and recorded. In this experiment, five samples were taken to increase the validity of the results in each experiment unit and all results were calculated using their mean. The collected data were analyzed using SPSS software.

Table 1
Analysis of variance for studied traits in canola under salinity stress

		Mean of squares											
S.O.V	df	Plant Height (cm)	Plant Dry Weight (g)	SPAD	Chlorophyll fluorescence	LWP (MPa)	Osmotic Potential (MPa)	Relative Water Content (%)	Na^+ ($mg.g^{-1}$)	K^+ ($mg.g^{-1}$)	K^+/Na^+	Proline content ($\mu mol.g\ fw^{-1}$)	Glycine Betaine ($\mu mol.g\ fw^{-1}$)
Replication	2	17.23^{ns}	0.276^{ns}	3.90^{ns}	0.0007*	0.005^{ns}	0.018^{ns}	84.18*	0.143^{ns}	26.50^{ns}	8.74^{ns}	8.34*	8.07*
Stress	2	395.45**	14.857**	18.09*	0.007**	0.196**	0.591**	149.05**	46.61**	589.79**	139.62**	45.33**	8.13*
Error	4	13.22	0.296	2.51	0.0001	0.004	0.014	11.03	0.109	18.28	4.08	1.15	1.04
CV (%)		5.77	5.44	4.01	3.28	5.36	10.76	4.74	7.50	18.58	28.59	21.46	23.92

^{ns}, *, and ** are in non-significant, significant at 5%, and significant at 1% probability levels, respectively.

Leaf samples from control and 300 mM NaCl (severe stress) groups were used for proteomic analysis, and leaf total protein was extracted by the method described in Damerval et al. (1986). The first and second dimension electrophoresis were performed by IPG strips and SDS-PAGE, respectively (Herbert, 1999). The staining was performed using commassie blue solution and finally, the repeated protein spots were identified and labeled by PDQuest software. The volume percentages of these repeated points that were quantified by the software were submitted to variance analyze to determine protein spots with significant response to stress (p≤05). Of the significant spots selected, spots whose Induction factor (IF) was greater than 2 or less than 0.5 were selected. A spot having an IF greater than or smaller than a unit indicates that it has shown an increase or decrease in expression under stress. Finally, after enzymatic digestion of the target protein spots, two-step mass spectrometry (MS/MS) was used to identify the spots. The mass spectrometer data were analyzed using MASCOT search engine and NCBInr database and results of mass spectrometry were used to identify target proteins.

Results

Analysis of variance and mean comparison of the study traits

Results showed a significant difference between the levels of stress for all traits evaluated (Table 1). Plant height and dry weight, which are part of morphological traits, significantly decreased under salinity stress (p≤0.01) (Table 1 and Fig. I). In this experiment, traits related to photosynthesis, i.e. chlorophyll fluorescence and chlorophyll index showed statistically significant decrease under stress conditions at p≤0.01 and p≤0.05 probability levels, respectively (Fig. I).

Analysis of variance showed that the effect of salinity on these traits was significant at 1% probability level (Table 1). The osmotic potential of the studied cultivars was significantly different and the highest osmotic potential was obtained at

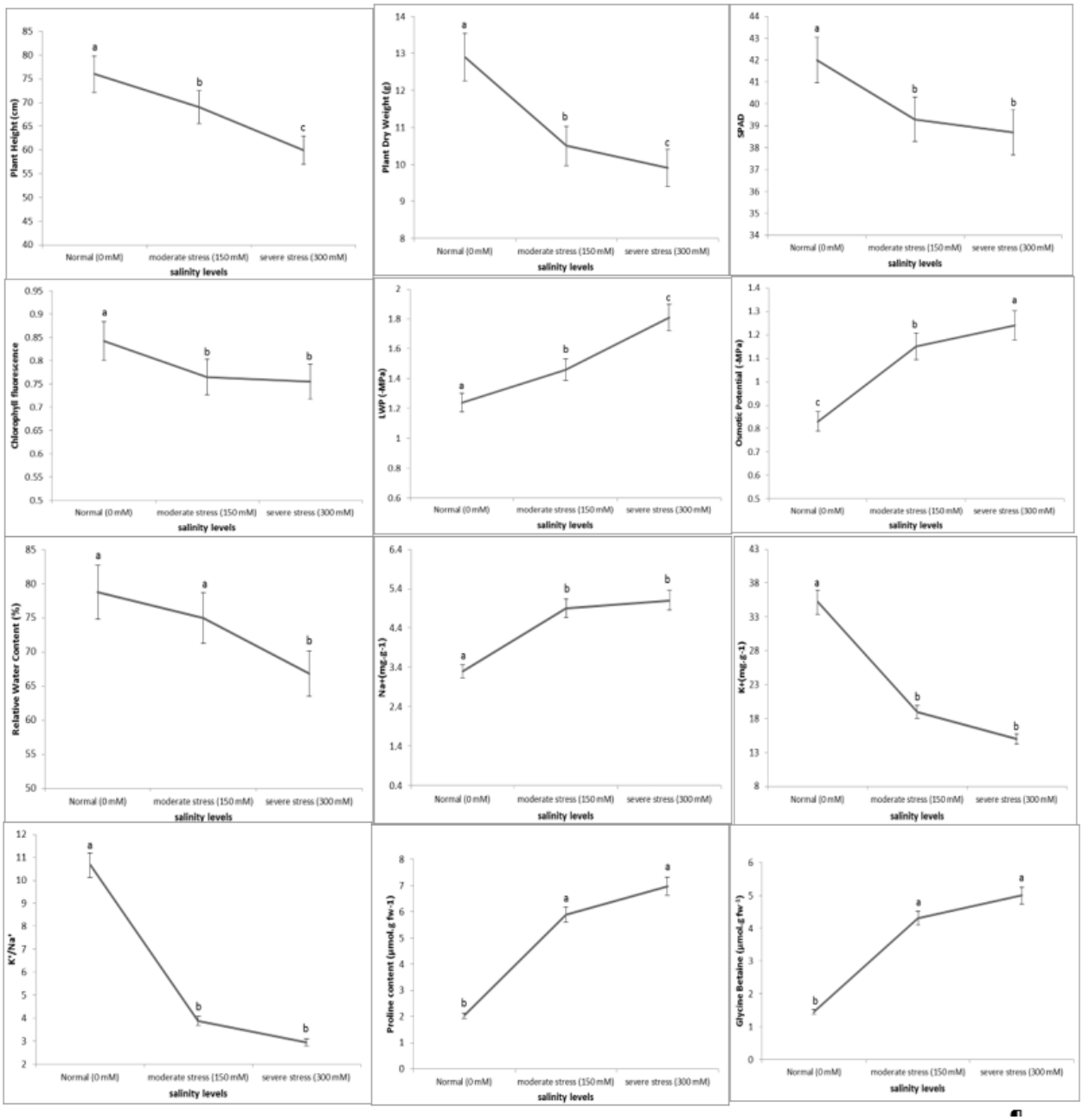

Fig. I. Effects of salinity stress (0, 150, and 300 mM NaCl) on the studied traits of Fornax cultivar; columns with the same letters are not significantly different at P≤0.05 level by Duncan's multiple range test.

obtained at the salinity level of 300 mM NaCl. On the other hand, the salinity stress reduced the leaf water potential from -1.24 MPa under control to -1.81 MPa under severe salinity (Fig. I). Also, relative water content of leaves decreased with increasing salinity levels and the differences between stress levels were significant at 1% probability level (Table 1 and Fig. I). The highest slope of leaf relative water content decreased between mild and severe stresses (Fig. I).

Salinity stress caused an imbalance in ionic concentrations (Na^+, K^+, and K^+/Na^+ ratio), so that with increasing NaCl concentration, Na^+ concentration increased significantly and reaching a peak at 300 mM level. The trend for K^+ was the opposite of Na^+ and the K^+/Na^+ ratio at the control level was higher than the salinity levels of 150 and 300 mM (Fig. I).

On the other hand, analysis of variance for proline and glycine betaine (Table 1) showed that

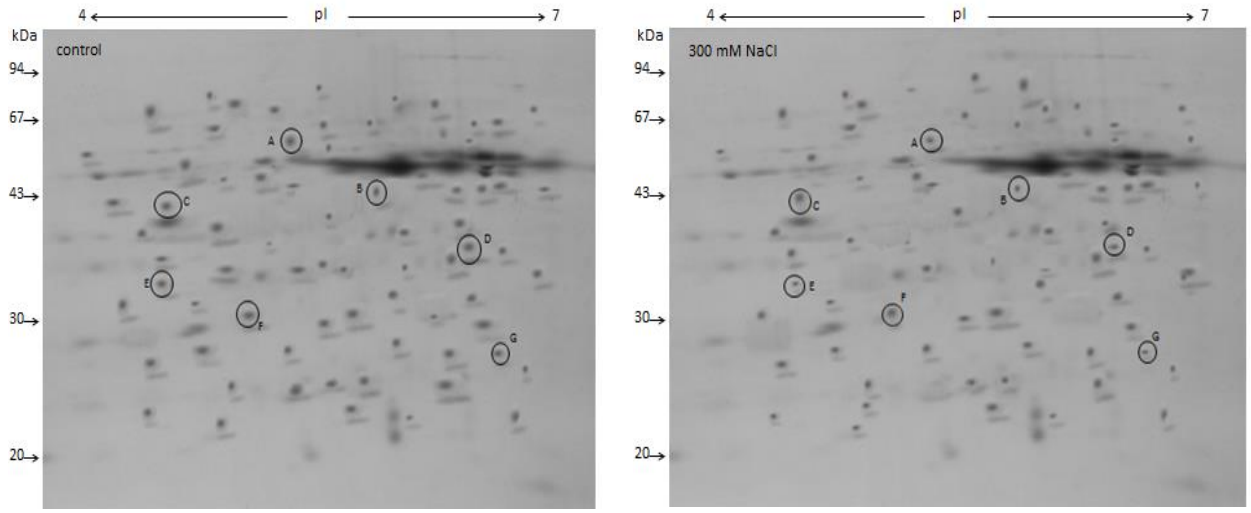

Fig. II. Reference gel of Fornax cultivar in control condition (left) and in severe stress condition (right) where responsive protein spots to salinity stress by numbers are marked

Table 2
Characteristics of 7 known protein spots in Fornax cultivar under salinity stress condition

Functional group of protein	Spot number	Experimental		Theoretical		name of protein	Accession number	Expression change
		MW	pI	MW	pI			
Calvin cycle	A	65.01	5.23	42.55	6.04	Sedoheptulose-1,7-bisphosphatase, chloroplastic	gi|1173347	Decrease
Assimilation of Nitrogen	B	43.56	5.75	47.41	5.11	Glutamine synthetase leaf isozyme, chloroplastic	gi|121340	Decrease
Antioxidant defense	C	41.53	4.48	23.6	5.8	Glotathione S-transferase	gi|5923877	Increase
Calvin cycle	D	38.96	6.25	47.33	6.03	Glyceraldehyde-3-phosphate dehydrogenase B, chloroplastic	gi|473912215	Decrease
Photo-reaction of Photosynthesis	E	35.31	4.34	23.10	5.90	Cytochrome b6-f complex iron-sulfur	gi|136707	Decrease
Antioxidant defense	F	30.05	4.95	17	5.37	Type 2 Peroxiredoxin	gi|473787383	Increase
hoto-reaction of Photosynthesis	G	26.52	6.41	29.3	8.69	Chlorophyll a-b binding protein 8, chloroplastic	gi|474121685	Decrease

production of both substances was affected by salinity stress, but glycine betaine level was significant at 5% probability level. In addition, the concentration of both substances increased as a result of salinity stress and with increasing stress level. The concentration of proline and glycine betaine at 300 mM salinity was about 6.98 and 5.00 µmol g fw^{-1}, respectively (Fig. I).

Proteome analysis

Two-dimensional analysis of electrophoresis pattern revealed that out of 104 protein spots, 7 protein spots were detected with significant difference and significant IF between control plants and severe salinity stress were detected in Fornax (Fig. II). According to the changes in the expression of protein spots, it can be concluded that most of the proteins in Fornax susceptible cultivar had reduced expression. Seven protein spots were identified by two-step mass spectrometry with significant differences between normal and stress conditions(Fig III). As shown in Table 2, according to their cellular function, these proteins were related to antioxidant defense, photosynthetic photolysis reaction, Calvin cycle, and assimilation of nitrogen. Among protein spots identified, wo protein spots (protein spots coded C and F) were related to antioxidant defense, two

protein spots were involved in photo-reaction of photosynthesis (protein spots E and G), two protein spots were involved in the Calvin cycle (protein spots A and D), and one protein spot was involved in nitrogen assimilation (protein spot coded B) (Table 2).

Discussion

A significant difference was found between the levels of stress for all traits evaluated. This is similar to the findings reported by Sairam et al., (2002). Francois (2007) found that salinity treatment decreased dry weight in both susceptible and tolerant canola cultivars, but the decrease was more in susceptible cultivar. Chlorophyll fluorescence analysis is an indicator of photosynthetic system and tolerance to environmental stresses (Schreiber et al., 1994). Similar to the present study, Takahashi and Murata (2008) found that chlorophyll fluorescence decreased under stress conditions. On the other hand, it has been reported that salinity stress and the resulting ionic changes along with a decrease in the hydraulic conductivity of water in the soil have a negative effect on photosynthetic yield of the plants (Khalid et al., 2015; Khan et al., 2009). Salinity treatment decreased chlorophyll index in both drought susceptible and tolerant canola cultivars, while this negative effect was more significant on the susceptible cultivars (Sairam and Tyagi, 2004; Hortensteiner and Krautler, 2011). Overall, in plants grown under saline conditions, photosynthetic activity decreases, thereby reducing their chlorophyll contents and chlorophyll fluorescence (Khan et al., 2009). This is also confirmed in the present study. Although plants vary in salinity tolerance, eventually salinity reduces their growth. This is mainly due to the decrease in photosynthetic capacity, which may be a consequence of the decrease in chlorophyll content following the decrease in the activity of enzymes effective in the synthesis of chlorophyll such as ALA-dehydrogenase (Hortensteiner and Krautler, 2011).

The effects of salinity on water relations, osmotic potential, leaf water potential, and leaf relative water content in this study are consistent with the reports of Pandey and Penna (2017). In general,

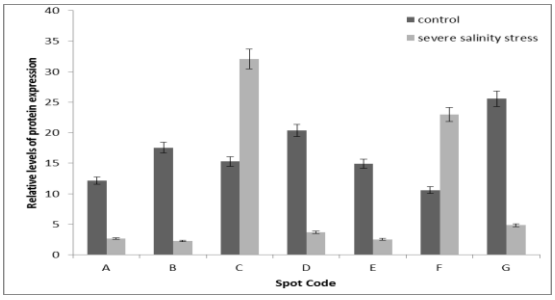

Fig. III. Relative levels of significant protein expression changes with significant induction factor under salinity stress conditions

reduction of leaf water potential and osmotic potential of leaf are mechanisms for plant survival when faced with water deficit stress (Chimenti et al., 2002). Negative effects of salinity on plant growth are attributed to the low osmotic potential of the soil solution (osmotic stress), special ionic effects (salinity stress), nutrient imbalances, or a combination of these factors. Under these conditions, leaf osmotic potential is negative and leaf water potential is reduced compared to the normal condition (Munns and Tester, 2008). Leaf relative water content is one of the most important physiological traits that decrease under salinity stress. Salinity stress disturbs water balance in plants and consequently, leaf relative water content decreases (Sairam and Tyagi, 2004). Numerous reports indicate that stress-sensitive cultivars maintain leaf relative water content less than tolerant cultivars (Morant-Manceau et al., 2004). On the other hand, the reduction of osmotic potential that leads to the preservation of the turgid pressure in leaves is usually caused by the increase and accumulation of soluble salts in plant cells (Munns and Tester, 2008).

In plants under stress, K^+ is transmitted to the leaves while Na^+ is distributed more evenly. Hence, the K^+/Na^+ ratio in plants under normal conditions is higher than in stressed plants and decreases with increasing salinity (Pandey and Penna, 2017). Many studies have linked ionic effects to salt stress tolerance (He and Cramer, 1993; Pandey and Penna, 2017). In this regard, it has been reported that the degree of salt tolerance is negatively correlated with Na^+ accumulation. This indicates an inverse relationship between tissue and cellular tolerance with Na^+ concentration under stress. High

concentration of this ion in leaves results in inefficiency of K^+ and inappropriate reaction of stomata. In other words, the high rate of Na^+ uptake competes with the uptake of other elements, especially K^+, which results in K^+ deficiency. The results showed that there is an ion transport mechanism for Na^+ that is against K^+ accumulation in Fornax. In plants under stress, reduced turgidity of the stomatal retaining cells can be induced by K^+ deficiency and therefore, selective uptake of this ion will lead to better regulation of stomatal cells and metabolism (He and Cramer, 1993). Low K^+/Na^+ ratio is an indicator of plant poisoning, because Na^+ disrupts the activity of K^+-dependent enzymes and eventually decreases growth (He and Cramer, 1993). Increased levels of sodium and the ratio of sodium to potassium have been reported in the shoots of plants under salt stress (Ashraf and Mc Neilly, 2004). The same reports indicated that the amount of potassium in the shoots under salinity is due to the antagonistic relationship between Na^+ and K^+. Therefore, the presence of Na^+ ion in the medium greatly prevents the absorption of K^+. This was clearly observed in the present study. Overall, the low cytosolic sodium concentration and the imbalance of the potassium-sodium ratio (K^+/Na^+) is one of the most important aspects of salt stress tolerance.

As cells are exposed to osmotic stress due to salinity and drought, adaptive solutions accumulate in them, thereby preserving cellular water content despite decreasing tissue water potential. Different species and varieties differ according to the type of solutions they accumulate (Saadia et al., 2012), and the osmolytes that play an important role in osmotic regulation depend on the plant species. Proline and glycine betaine are adaptation solutions that accumulate in response to osmotic stress, and accumulation of these solutions is an important adaptation reaction. Proline accumulation occurs under salinity stress more than other amino acids, which may contribute to osmotic regulation and possibly maintenance of plant enzymatic activity (Khan et al., 2009). Various reports have shown that proline content is one of the most important criteria for stress tolerance in most plant species, and lower proline content was reported in salinity sensitive as compared with resistant cultivars (Verslues et al., 2007). Similar to proline, production of glycine betaine and its increase was observed under salinity stress. Although the level of this substance increased under salinity stress and with its increase, in some previous experiments, despite its increase under stress, this increase was not statistically significant (Khalid et al., 2015). This trend can be attributed to variations in experimental conditions or actual differences between species and plant genotypes in response to salt stress (Khalid et al., 2015).

The response of proteome in Fornax cultivar under salinity stress varied as its expression decreased or increased consumption of proteins with intracellular activity, and further decrease in these proteins' levels under salinity stress seems to be effective in susceptibility of this cultivar. These results are consistent with the results of Yildiz et al. (2015). In general, responsive proteins in Fornax cultivar were classified into antioxidant defense, photosynthetic related proteins, and nitrogen assimilation. The presence of glutathione S transferase I (GST) proteins (protein spot with C code) indicates the induction of oxidative stress under salinity stress. Most plant GSTs are cytosolic enzymes (Dixon et al., 2002) that are seen as homo- or heterodimers (Edwards et al., 2000). In practice, GSTs are glutathione-dependent detoxifying enzymes that blend glutathione with various natural products. The blended glutathione is then transferred to the vacuoles for subsequent metabolism involving sulfur (Edwards et al., 2000). Researchers have found that glutathione transferase levels under stress in tolerant cultivars increase and increase less in susceptible cultivars (Kausar et al., 2013), which is consistent with the results of this experiment. Increasing the amount of glutathione transferase during stress is to counteract the adverse effect of ROS (Dhindsa, 1991). On the other hand, the production of peroxyredoxin (protein spot with F code) which has antioxidant activity has been reported in many studies due to abiotic stresses (Samaj and Thelen, 2007). Peroxideredoxin proteins are widely found in tissues and mitochondria, peroxisomes, and cytosol. The cellular location of these proteins indicates that they have an important antioxidant role in the cellular organs that are the major

source of ROS. In addition to antioxidant activity, these proteins are active in controlling cellular signals. The end of these proteins contains cysteine, which is oxidized polyethylene sulfinic acid to react with peroxide. In fact, the activity of superoxide dismutase enzyme converts ROS to H_2O_2, and reduces peroxidase type 2, H_2O_2 molecules, and in many studies the peroxideredoxin has been reported to react to stress as a responsive protein under stress (Joseph and Jini, 2010). The active presence of this protein in Fornax cultivar (protein spot with F code) showed the role of this protein in reducing the adverse effect of salinity. Overall, the presence of proteins involved in antioxidant defense and reduction of other proteins in stress conditions helps to prevent further damage to the susceptible cultivar Fornax under salinity stress.

Chlorophyll-binding proteins are made by precursor molecules in the cytoplasm and must be transferred to the chloroplast and inserted into the thylakoids and also embedded in the chlorophyll structure (Heide et al., 2004). These proteins carry out several important tasks, such as receiving light, transmitting energy, and storing pigments. The primary task of proteins bound to chlorophyll a/b is to absorb light and stimulate energy transfer to photochemical reaction centers (Bassi et al., 1997). In some cases, plants are overwhelmed with photosynthetic need; therefore, they are lost and destroyed by these photoreceptor proteins to prevent optical inhibition and damage to the photosynthetic apparatus. In addition, it is believed that chlorophyll a-b binding proteins are active in storing pigments (Bassi et al., 1997). In other words, the light energy absorbed by the chlorophyll a-b binding proteins (protein spot with G code) is used to activate light-dependent oxidation and release the oxygen molecule. Therefore, it seems that the damage to this part is high in Fornax cultivar. On the other hand, the chemical decomposition of water by photolysis is performed at the OEC in the photosystem II reaction center (Heide et al., 2004).

Subunits of the PSII complex are proteins that are involved in photosynthesis and are known as OEC proteins, which also play a role in the stability of the PSII complex (Ifuku et al., 2008). Disruption of these proteins causes light damage to photosystem II (Takahashi and Murata, 2008). The electrons isolated from water molecule by photosystem II are eventually transferred to $NADP^+$ via photosystem to produce NADPH. This process is called linear electron transfer. Simultaneously with this process and as a result of the electron transfer from the Cytochrome b6-f complex iron-sulfur (Cyt b6/f) (protein spot with E code) the proton gradient is created in the thylakoid membrane (Allen, 2003). Reports have shown that decreasing Cyt b6/f complex results in a decrease in electron transfer ratio and carbon dioxide production ratio (Ruuska et al., 2000). Overall, the reduced or no expression of these proteins in the susceptible cultivar Fornax, which includes protein spots G and E, disrupts the activity of photosystem II and ultimately reduces the efficiency of the light response. These results indicate that salinity stress, an important component of the photosynthetic machinery, i.e. the oxygen spin and complex of photosystem II, is strongly influenced and hence contributes to leaf senescence and possibly cell death.

On the other hand, in terms of function, Rubisco proteins catalyze the carbon stabilization (carboxylation) reactions that take place in the Calvin cycle of photosynthetic plants. In this process, ribulose 1, 5-bisphosphate (RuBP), a five-carbon compound, acts as a carbon dioxide molecule receptor and forms an unstable six-carbon compound. This unstable compound is immediately decomposed into two molecules of 3-carbon triphosphoglycerate (3PGA) (Andersson and Backlund, 2008). The end product of this reaction is 3PGA carboxylation, which is phosphorylated by ATP and forms 1, 3-bisphosphoglycerate and ADP. This reaction is catalyzed by cytosolic 3-phosphoglycerate kinase. This reaction is one of the two reactions that occur in the second phase (reduction phase) of the Calvin cycle, which is subsequently hydrogenated 1,3-bisphosphoglycerate by hydrogen capture from NADPH (Macdonald and Buchanan, 1997). Glyceraldehyde phosphate dehydrogenase (GAPDH) plays a key role in reducing glycerate-3-phosphate to glyceraldehyde-3-phosphate. Glyceraldehyde-3-phosphate is not only a

photosynthetic product, but also acts as a 5-phosphate ribulose precursor (Ye et al., 2013). GAPDH B (protein spot with D code) has been identified and reported by Yang (2008). This protein affects the efficiency of the Calvin cycle in the light response and accumulates photosynthetic and plant products (Pillai et al., 2002). On the other hand, in the third phase of the Calvin cycle, RuBP molecules are rebuilt and the Calvin cycle starts from the beginning. The resuscitation phase is characterized by a series of enzymatic reactions that convert triosphosphate to RuBP (Macdonald and Buchanan, 1997). Some of the enzymes mediated in this phase include sedoheptulose-1,7-biphosphate (protein spot with A code) and fructose 1,6-biphosphate aldolase, the first enzyme identified in this experiment. These two enzymes catalyze the reaction together resulting in the formation of ribulose-5-phosphate. Then ribulose-5-phosphate phosphorylates to form RuBP. To complete the cycle, RuBP as the target material is used by RuBisCo, and again the first phase, which is carbon stabilization, is performed. Some of the thiophosphates produced in the Calvin cycle are used for sugar and starch biosynthesis (Tamoi et al., 2005). Overall, the yield of photosynthesis-related proteins detected in Fornax showed a decrease in the photosynthetic yield of this cultivar under salinity stress.

Nitrogen is a key element in reproduction and growth, and it is essential for the production of proteins and nucleic acids. Plants can store high amounts of nitrogen in enzymes involved in carbon fixation such as Rubisco. The main pathway for inorganic nitrogen production and remobilization in plants at biochemical level has been demonstrated. GS (protein spot with B code) is a key enzyme in this pathway which catalyzes ammonium-dependent adenosine triphosphate to the δ-carboxyl glutamate group for glutamine formation, in contrast to glutamate synthase, which converts glutamine and 2-oxoglutarate binds to two glutamate molecules (Stéphanie et al., 2009). It has been reported that in response to drought and salinity, the abundance of GS polypeptides and their activity reduce, especially in susceptible cultivars (Santos et al., 2004), also consistent with the results of this experiment.

Overall, according to the results of this study, due to the role of functional proteins and their lower expression in susceptible cultivar Fornax, this cultivar does not have a proper molecular mechanism under salinity stress induced by sodium chloride. In fact, among various metabolic pathways, this cultivar only employs antioxidant defense system under stress conditions.

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