

Proteomics analysis of salt stressed Sunflower (*Helianthus annuus*)

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ABSTRACT

Sunflower (*Helianthus annuus* L , is the most important oilseed crop, where salinity is an increasing problem. Sunflower is moderately sensitivity. The physiological results showed that the effect of sodium chloride was significant and highly significant decreased gradually for all varieties and all treatments, in seeds germination (reached to 0% approximately in all varieties from 200 mM), roots lengths with a difference of 22 cm from the control to 250 mM in Teddy bear variety. Concerning the biochemical effect, the proteomic analysis using 2D-gel electrophoresis was limited to just for Teddy bear variety where observed the existence of spots in plant control not observed in salt treated sample with 250 mM and appearance others in sample treated not observed in the control sample. The SDS-PAGE for four untreated varieties (Sakha53, Giza 102, Abendsoone and Teddy bear) showed (76.92%) of polymorphism, while for untreated and treated varieties with 100 mM and 250 mM recorded (65%). The cluster analysis for untreated varieties included the Egyptian varieties Sakha 53 and Giza 102 with (80%) similarity and 50% between Abendsoone and Teddy bear varieties, for untreated with treated varieties recorded (72.7%) between all controls and 250 mM, while noted (25%) between Abendsoone variety and 250 mM, while The effect of salt were positive with appearance of two bands (in 250 mM before and after 18.5 kDa for Abendsoone variety) not observed in control.

Key words: Sunflower, Salinity, sodium chloride, 2D gel electrophoresis, SDS-PAGE, varieties.

INTRODUCTION

Biotic and abiotic environmental factors may constitute stresses that affect plant growth and yield in crop species. With a focus on ionic stress exerted by the presence of sodium⁵. Plants all over the world are subjected to multitude of stresses throughout its growth period. The major environmental factor that currently reduces plant productivity is salinity²¹. Salty soils extensively exist in arid and semi-arid climate regions of the world and cause salt stress in plants¹⁶. Sunflower (*Helianthus annuus* L.), is a major source of vegetable oil in the world. Worldwide production of sunflower has increased, is one of the 67 species in the genus *Helianthus*, diploid ($2x=34$)³, is a model system for the genomic studies of the family Asteraceae²⁴. The sunflower is valuable from an economic, as well as from an ornamental point of view. Every part of the plant may be utilized¹⁰. Proteome analysis is a direct measurement of protein in terms of their presence and relative abundance³⁶. The overall aim of proteomic study is the characterization of the complex network of cell regulation. Neither the genomic DNA code of an organism nor the amount of mRNA that is expressed for each gene product (protein) yields give and accurate picture of the state of a living cell²⁰. High-resolution two-dimensional electrophoresis (2-DE) allows for the quantitative and qualitative separation of complex proteins mixtures usually extracted from cellular organisms. Sample preparation is an important step and is absolutely essential to allow reliable results⁶. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is most widely used due to its validity and simplicity for describing genetic structures of group of plants, that is used to

distinguish between genotypes¹⁷. The present study aim is to screening of salt stress effects on Sunflower (*Helianthus annuus*) varieties and assessing the genetic diversity using to identifying proteins and quantitative their expression levels under different concentrations of sodium chloride, determine their modification states, localizations, interaction partners and assign their functions, finally determine the percent of polymorphic and the expected heterozygosity values.

MATERIALS AND METHODS

Plant material and salt treatment

Healthy seeds of six varieties of *Helianthus annuus L* were used in this study: Sakha 53, Giza 102, Abendsonne, Teddy bear, Gigante and White Hassawi, taken from different sources, exposed to five concentrations of sodium chloride: 50 mM, 100 mM, 150 mM, 200 mM, 250 mM.

Measuring the content of plant biochemical substances:

Two-dimensional (2-D) gel electrophoresis:

Protein extraction:

Protein extraction protocol combined two procedure (TCA/acetone) precipitation¹⁵ with SDS/phenol extraction³⁵. Leaves (0.5g) was ground in a mortar and pestle in the presence of liquid N₂. Ground tissue powder (approximately 0.2 g per tube) was placed in 1.5–2.0 ml capacity microcentrifuge tubes, suspended in 10% TCA/acetone with 0.07% b-mercaptoethanol (b-ME), mixed, and centrifuged for 3 min at 16,000g and 4 °C. This step was repeated twice, followed by washing the pellet with ice-cold acetone containing 0.07% w/v b-ME twice. The pellets were air-dried, mixed with 0.4–0.8 ml (per 0.1 g plant material) of SDS buffer containing 30% sucrose, 2% SDS, 5% b-ME in 0.1 M Tris–HCl (pH 8.0), Tris-buffered phenol, and vortexed thoroughly for 30 min at ambient temperature, centrifuged at 16,000g for 5 min at 4°C. The upper phenol phase was then mixed with four volumes of 0.1 M NH₄OAc in MeOH–H₂O (4:1, v/v) and incubated overnight at -20 °C. It was centrifuged (14,000g, 5 min, 4°C) and the pellet was then washed once with 0.1 M NH₄OAc in MeOH–H₂O (4:1, v/v) followed by an (500 µl) acetone–H₂O (4:1, v/v) washing. The pellet was air-dried and dissolved in 1% SDS buffer.

Protein separation

The technique relies on one isoelectric focusing step, which separates protein on the basis of isoelectric point, followed by a second separation based on denaturing SDS gel electrophoresis, which resolves proteins on the basis of molecular weight. The 2-DE of total proteins was performed via capillary isoelectrofocalization (IEF). In the first dimension the ZOOM IPG Runner system were used, pH 3–10 non-linear IPG strips, 200 µl of protein sample were added in the 200 µl rehydration buffer (8M urea, 2% CHAPS, 20mM DTT, 1% ampholites and bromophenol blue). Samples were first applied in the mode of in-gel RB (equilibration buffer) of the IPG strips and passive rehydration was carried out overnight at room temperature, for 20 min at 200 V, 15 min at 450 V, 15 min at 750 V, and 1 h at 2000 V. After focusing and prior to the 2D analysis strips placed in the 10 ml equilibration buffer according to Görg et al⁸ (Tris HCl 0.5 M pH 6.8, SDS 10%, Glycerol 10%, (DTT) 154.2 mg, Bromophenol bleu 25 mg) for 15min. In the second dimension the strips were then transferred onto vertical slab and covered with 0.5% agarose. Protein molecular weight marker (Perfect Protein Markers: 15-150 kDa) was applied to the well provided on the gel for calibration of the molecular weight. The gels were run with an electrophoresis buffer SDS running buffer (3g tris+14.4 g glycine + 1g SDS and 1l DDW) and electrophoresis was performed at 200 V for 35–40 min. Gels were stained with Coomassie staining CBB G-250 for 1h and destained with 50% (v/v) methanol and 10% (v/v) acetic acid after gel images.

SDS-PAGE

Soluble protein content:

Soluble protein content was estimated following the method of Bradford⁴. With the help of pre-cooled mortar and pestle, approximately 1 g of fresh leaf tissues was homogenized first in liquid nitrogen. Then three volumes of extraction buffer (50 mM Tris–HCl (pH 7.5), 1 mM PMSF, 2 mM EDTA, 1 mM 2-mercaptoethanol,) was added followed by vortexing for 10 min. The homogenate was centrifuged for 25

min at 14 000 rpm at 4°C. The supernatant was collected and mixed with three volumes of cold acetone. Then samples were kept at –20°C for 30 min followed by centrifugation for 15 min at 14000 rpm at 4°C . The supernatant was then removed, and the pellets were air-dried at room temperature. Thereafter, the pellets were resuspended in a small volume of extraction buffer and centrifuged for 5 min at 14000 rpm at 4°C. Finally, the supernatant was prepared for protein assay. Absorbance was then recorded at 595nm on UV-vis spectrophotometer. The protein content was expressed as mg/g/ fw.

Preparation and running of the SDS-PAGE gel

Denaturing electrophoresis was performed according to Laemmli¹⁹, in 10% acrylamide gel with SDS and used without further purification in the hoefler vertical slab unit. 10% separating gel containing 375 mM Tris-HCl (pH 8.8), 0.1% (w/v) SDS, 0.05% (w/v) ammonium persulfate and 10 µl TEMED was used for resolving the polypeptides. The separation gel were poured in to the gradient maker chambers connected to the casting stand with two glass sandwich. whereas a 5% stacking gel (were added after 45 min), containing 125 mM Tris-HCl (pH 6.8), 0.1% (w/v) SDS, 0.05% (w/v) ammonium persulfate and TEMED was used to concentrate (stack) the polypeptides and added in the last for each gel. The comb was slotted immediately and added alcohol (70 %) for removing bumbles. After drying of gel (30 min) and the comb was taken out, it putted in the running buffer (3g tris+14.4 g glycine + 1g SDS+ distilled water for making total volume 1 litre).The sample buffer contains the following ingredient: 40 µg protein were mixed with equal volumes of solubilizing buffer [62.5 mM Tris-HCl(pH 6.8), 20% (w/v) glycerol, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol and 0.01% bromophenol blue] and heated for 4 min at 95°C. Electrophoresis was accomplished at 20 mA for 1 h. The gels were stained with 0.25% Coomassie Brilliant Blue R-250 for 2 h and destained with 50% (v/v) methanol and 10% (v/v) acetic acid until the background was clear. The gels were photographed and scanned using a densitometer .

Statistical analysis was conducted with MVSP 3.1 (MultiVariate Statistical Package) programme for protein and DNA. Bands were scored visually and recorded as presence (1) or absence (0) of bands. Similarity coefficients were used to construct the dendrogram using the unweighted pair group method with arithmetic means (UPGMA)

RESULTS

Physiological analysis

Effect of salt on germination percentage:

The concentrations of sodium chloride (50mM, 100 mM,150 mM ,200 mM,250 mM) were tested on growth of seeds of *Helianthus annuus L* compared with control, Figure (1) showed that percentage of germination decreased with increasing concentrations where in Sakha 53 the percentage of germination was 86.66 % in control decreased gradually until you reach 6.66 % at 250 mM, in Giza 102 percentage of germination was 86.66 % decreased from 150 mM (20%) to 250 mM (0 %) in Abendssoone was 60 % at control and 46.66 % in Teddy bear and stood at zero in both variety from 200 mM whereas in, Gigante reached to 0 % from 150 mM while the percentage of germination was 40 % in the control in Hassawi 46.66 % decreased until they reached the 6.66 % at 150 mM and 0 % at 250 mM. Percentage of germination was significant and stood at zero in most of varieties at 250 mM Table 1.

Table 1: Germination percentage

Variety	Control	50 mM	100 mM	150 mM	200 mM	250 mM
Sakha53	86.66	73.33	66.66	66.66	33.33	6.66
Giza102	86.66	66.66	33.33	20	6.66	0
Abendssoone	60	46.66	20	13.33	0	0
Teddy bear	46.66	20	13.33	6.66	0	0
Gigante	40	20	6.66	0	0	0
White Hassawi	46.66	20	13.33	6.66	6.66	0

Root length

Root length of *Helianthus annuus L.* vary between variety, it was significant decreased with application of salt treatment (Figure 2) at (100mM,150mM) and highly significant at (200 mM,250mM) in Sakha 53 compared with control (17.26 cm) and Giza 102 (15.33cm) and significant in abendsoone from 150 mM to 250 mM compared with control (16.66 cm), and highly significant in Hassawi at 250 mM decreased compared with control (7.86 cm), in Teddy bear it was significant at (50 mM, 100 mM, 200mM) and highly significant at 250 mM decreased with control (27.30 cm), in Gigante it was significant at (100mM, 150mM, 200mM, and 250 mM) compared with control which is 6.30 cm (Table2).

Table 2: Root length

Treatments Mm	Parameter (Mean \pm SD)					
	Variety					
	Sakha 53	Giza 102	Abendsoone	Teddy bear	Gigante	Hassawi
C	17.26 \pm 0.873	15.33 \pm 1.011	16.66 \pm 1.607	27.30 \pm 7.502	6.30 \pm 0.360	7.86 \pm 0.650
50	16.66 \pm 1.040	14.40 \pm 0.500	13.53 \pm 1.234	17.30 \pm 2.151*	6.00 \pm 0.854	7.70 \pm 0.916
100	15.06 \pm 0.152*	12.26 \pm 0.776*	16.30 \pm 1.539	15.93 \pm 2.267*	3.90 \pm 1.493*	6.50 \pm 0.871
150	14.36 \pm 0.568**	11.16 \pm 1.258**	10.26 \pm 2.059*	10.10 \pm 2.253**	3.43 \pm 0.503*	5.53 \pm 0.493**
200	10.90 \pm 0.964***	10.46 \pm 0.450***	8.73 \pm 2.853**	9.86 \pm 0.493**	2.76 \pm 0.757 **	5.03 \pm 0.321**
250	4.83 \pm 0.763***	5.00 \pm 1.345***	6.76 \pm 2.653**	5.30 \pm 0.624***	3.03 \pm 1.594**	4.43 \pm 0.351***
LSD at 5 %	1.398	1.699	3.693	6.153	1.454	1.147
LSD at 1 %	1.958	2.379	5.178	8.627	2.037	1.608

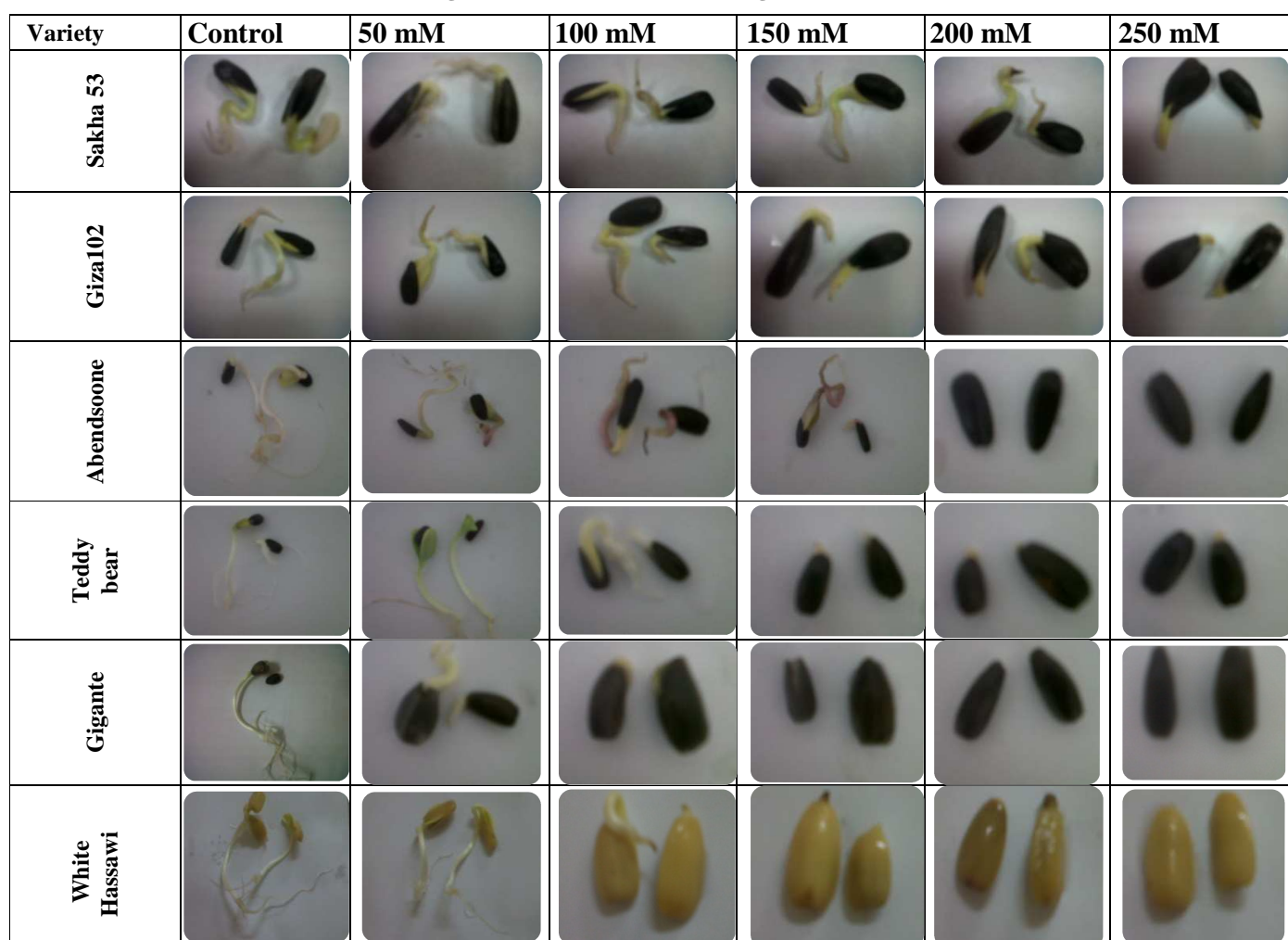
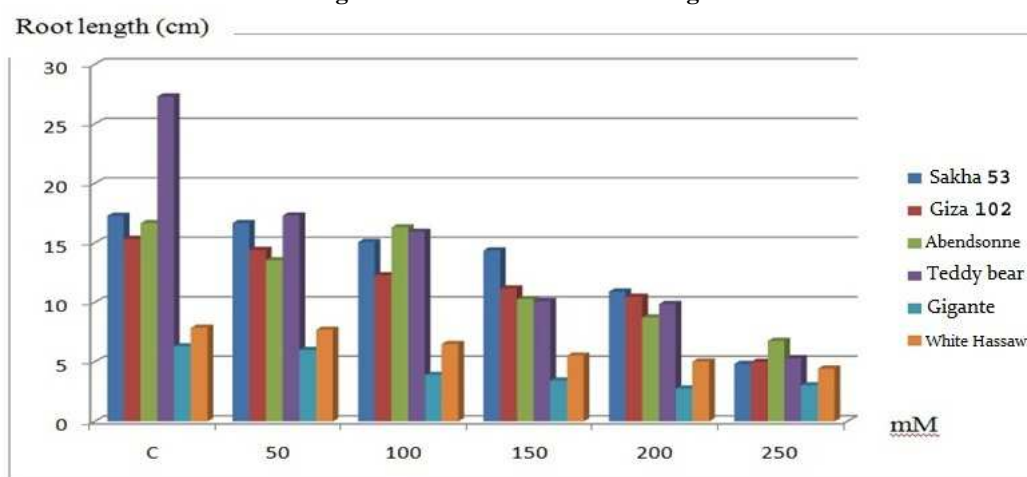
Figure 1: Effect of salt on seeds germination

Figure 2: Effect of salt on root length



Protein

D gel electrophoresis:

Analysis of 2-D gel electrophoresis tested for Teddy bear variety, on formed of spots, Figure (3) showed 2-D gel obtained from leaf proteins of Teddy bear variety from combination of two procedure (TCA/acetone) precipitation with SDS/phenol extraction. Plants served as control and spots on the right of the Figure (A) between molecular weight 15-35 KDa were not observed in salt treated sample with 250 mM but in Figure (B) plants were exposed to salt treatment. Spots on the left of the Figure between 25-35 KDa were not observed in the control sample.

SDS-PAGE gel electrophoresis:

Figure (4) showed gel sliced for protein that gave clear bands, for Sakha 53, Giza 102, Abendsoone and Teddy bear varieties.

From forty bands regrouped controls and treatments (100 mM and 250 mM), (26) bands were polymorphic and (14) bands monomorphic, thus the polymorphism from total protein results were (65%). From the table (4) The lowest similarity matrix (0.25) recorded between Abendsoone variety (control) and the concentrations 250 mM, while the highest value (0.909) recorded between the control of Sakha 53 and the concentrations 100 mM and between these values ranged the convergence degrees of remaining samples.

The cluster analysis Figure (5) showed that Abendsoone variety was separated in a single branch and a cluster regrouped controls of four varieties and the concentrations 250 mM, while separated Teddy bear in a single branch, similar for Giza 102 and other cluster regrouped concentrations 100 mM with Sakha 53 variety.

Figure (4) showed appearance of bands in varieties treated not founded in controls (two bands in 250 mM before and after 18.5 kDa in Abendsoone variety) and disappearance of bands in all treated varieties.

DISCUSSION

Physiological effect of sodium chloride:

The response of *Helianthus annuus L.* plants to different concentrations of NaCl was investigated in the present study. The effect of NaCl were positively in seed germination, root in comparison with control; decreased with increasing concentrations. Varieties (Sakha 53, Giza 102, Abendsoone and Teddy bear) showed the best results; the difference between the control and the last concentrations (250 mM) were very large, while the two other varieties (Gigante and White Hassawi) results had not the same degree of effect; there was no significant difference between the control and 250 mM, in addition to the difficulty of distinguishing between different concentrations, it is possible explained by the quality of seeds, also differences in seeds germination.

Salinity affects germination in two ways: the first one there may be enough salt in the medium decrease the osmotic potential to such a point which retard or prevent the uptake of water necessary for mobilization of nutrient required for germination Figure (1). The second the salt constituents or ions may be toxic to the embryo.

The present results corresponded to these of Rahman *et al*²⁸, that germination was directly related to the amount of water absorbed and the delay in germination to the salt concentration of the medium. The salt tolerance of plants varies with the type of salt and osmotic potential of the medium .

Results thus indicate that salt can cause ionic stresses, Inside the cell, largely as Na⁺ (and Cl⁻) inhibit metabolic processes including protein synthesis. “Na⁺ can rise to toxic levels in older leaves, causing them to die. This reduces the leaf area available for photosynthesis and so the plant cannot sustain growth or crop yield²⁴. Reductions in the rate of leaf and root growth Table (2) are probably due to factors associated with water stress rather than a salt-specific effect. The cause of the injury is probably due to the salt load exceeding the ability of the cells to compartmentalize salts in the vacuole. Salts then would rapidly build up in the cytoplasm and inhibit enzyme activity. Alternatively, they might build up in the cell walls and dehydrate the cell²⁵.

Salt stress reduces the plant's ability to take up water, and this leads to reduction in growth. This is the osmotic or water-deficit effect of salt stress. Both cellular and metabolic processes involved in osmotic stress due to salinity are common to drought. The rate at which new leaves are produced depends largely on the water potential of the soil solution, in the same way as for a drought-stressed plant. Salts themselves do not build up in the growing tissues at concentrations that inhibit growth, as the rapidly elongating cells can accommodate the salt that arrives in the xylem within their expanding vacuoles. So, the salt taken up by the plant does not directly inhibit the growth of new leaves²⁵.

Most of the literature indicates that plants are particularly susceptible to salinity. Examples are found in Wheat, when Al-maskri *et al*² in lettuce recorded that, plant fresh weight, shoot fresh weight, shoot dry weight, shoot dry matter percentage, root fresh weight, root dry weight, root dry weight percentage, leaf area and leaf area index were significantly affected by salinity levels.

Oxidative stress is initiated by reactive oxygen species (ROS) (are chemically reactive molecules containing oxygen) such as superoxide radical (O⁻²), hydrogen peroxide (H₂O₂) and hydroxyl (OH⁻) radical are also produced during salinity stress, and are responsible for the damage to membranes and other essential macromolecules such as photosynthetic pigments, proteins, DNA and lipids, ROS scavenge by various antioxidant enzymes such as catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO), which increased during salt stressed. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generates and transmits signals and instigates biochemical changes that adjust the metabolism accordingly⁸.

Biochemical effect of Sodium chloride

Proteomic analysis:

The protein profiling or proteomics is another approach which has been used for study of related changes during stress. Analysis of proteins from control and stressed samples on 2D gel electrophoresis can provide information about new proteins as well as increased or decreased amount of proteins in stress condition.

Preparation of adequate protein samples for 2-DE analysis is the most critical step for any proteomics study. Proteins should be denatured, reduced and solubilized or rehydrated in order to obtain a complete disruption of their intra- and intermolecular secondary bonds and, hence, to assure that each spot in the gel represents an individual polypeptide. Moreover, proteins extracted from plant tissues are typically more difficult to resolve by 2-DE than those from other organisms. This is due to the abundance of interfering compounds, particularly when working with recalcitrant tissues from woody plant species. Removal of those compounds that can interfere in electrophoretic separation becomes a crucial matter for sample preparation. Salts, polysaccharides, pigments, nucleic acids, polyphenols and other secondary metabolites can cause vertical and horizontal streaking, smearing and reduced number of distinctly resolved protein spots in 2-D gels^{34,33}.

Reducing number of spots means proteins reduced from leaves; this is also because of their migration to the flower, proteins as FT-GFP (fusion protein -green fluorescent protein) moved from leaves through the phloem cells to the apical tissue, and was then unloaded from the phloem cells into the apical meristem cells, where it activated flowering³¹.

Pareek *et al*²⁵ suggested that stress proteins could be used as important molecular markers for the improvement of salt tolerance using genetic engineering techniques. However, proteins produced under salt stress are not always associated with salt tolerance; consequently, using proteins as a salt tolerance indicator depends on the nature of the plant or cultivar.

Plants reveal significant differences in their abilities to cope with salinity. It has been proposed by Inan *et al*¹² that generally, three major factors could determine the tolerance of plants to extreme environmental conditions (abiotic stresses) at molecular level: first genomic level: tolerant plants may possess some unique stress-responsive genes which are absent in susceptible plants (differences at genome structure level). Second transcriptomic level: tolerant plants reveal altered regulation of gene expression of important stress-responsive genes than susceptible plants (qualitative and quantitative differences at gene expression level). Third proteomic level: proteins involved in stress response reveal an altered activity in tolerant plants than in susceptible ones (differences in protein structure and activity level). To the molecular levels listed above, metabolomic and physiological (functional) levels can be added.

As shown in Figure (3), proteom spots were not present or absent in the control compared to their presence in Teddy bear variety at concentration 250 mM and disappearance of spots founded in the control and not in the sample treated; means that three protein spots showed a variety-dependent expression in the absence of salinity stress, with a total of six spots more abundant in leaves of the tolerant; appearance spots may be linked with salt tolerance, while disappearance of others may be the gene expression is altered under NaCl saline conditions²⁶.

The proteins were grouped into four categories. Proteins in the first category followed an expression pattern characterized by a genotype-specific expression, which was not significantly affected by salt stress treatment. Proteins in the second category were significantly more highly expressed under stress conditions in at least one genotype. The last category comprises spots that were regulated in an opposite manner between genotypes, in this point this study need identification of proteins using MALDI-TOF mass spectrometers. Similar results by Witzel *et al*³⁷ in barley conducted that twenty-four protein spots showed a cultivar-dependent expression in the absence of salinity stress, with a total of 10 spots more abundant in roots of the tolerant. Morex line cultivar, and 14 spots more highly expressed in the sensitive Steptoe line cultivar. Overall, the expression of 23 protein spots was affected by salinity treatment, while the abundance of three spots were not affected by the treatment. Based on the expression profiles under salt stress conditions, were two spots in the first category identified as late embryogenesis abundant protein and a lactoylglutathione lyase. Proteins in the second category; those were lipoxygenase 1, *S*-adenosylmethionine synthase 1, peroxidase, (1–3)- β -glucanase, and a carboxymethylenebutenolidase-like protein. The largest group contained proteins that were down-regulated in at least one genotype upon stress treatment, and these were 6-phosphogluconate dehydrogenase, a probable nicotianamine synthase 7, F23N19.10 (a stress-inducible protein), a putative monodehydroascorbate reductase (MDAR), a putative nuclear RNA-binding protein A, lactoylglutathione lyase, a poly(A)-binding protein, catalase 1, the iron deficiency-specific proteins IDS2 and IDS3, fructokinase 2, the iron deficiency-induced proteins IDI1 and IDI2, glutathione *S*-transferase (GST) F5, and a 23 kDa jasmonate-induced protein. Spot 1747 was the product of barley EST TC137024, a gene lacking any significant homologue in other plant species. The last category identified as a probable L-ascorbate peroxidase 7. According to their functional annotation, most protein spots identified in the analysis are part of the oxidative stress responses, with eight protein spots functioning in redox regulation. The remaining proteins are involved in protein synthesis, primary and secondary metabolism, desiccation tolerance, and disease/defence-related processes.

SDS-PAGE

The present study used SDS-PAGE technique was employed for analysis of leaf proteins diversity in four *H. annuus* varieties (Sakha 53, Giza 012, Abendsone and Teddy bear) as shown in Figure (4). The

polymorphism of protein for untreated varieties were (76.92%). SDS-PAGE analysis provided strong basis for the discrimination of genotypes on the basis of specific polypeptide fragments.

Also has been successfully applied in many different plant species to estimate genetic diversity and phylogenetic relationship among genotypes, whereas the polymorphism were in wheat 5- 84.0% polymorphism among 10 genotypes³⁰. Similarly, Khan *et al*¹⁷ observed 0-60.0% genetic polymorphism with in twenty genotypes of walnut. Using SDS-PAGE, Inamullah *et al*¹³ have showed 0-80% polymorphism among eleven genotypes of *Oryza sativa*. In *Capsicum*, 0-100% polymorphism was observed in nineteen genotypes¹, while in Kabuli Chickpea genotypes significant polymorphism was reported¹⁰.

SDS-PAGE protein banding pattern for untreated varieties, grouping in one major cluster Figure (4.16), the Egyptian varieties (Sakha 53 and Giza 102) recorded (80%) similarity Table (4.12), indicate moderately low variability; which may be attributed to their geographic origin, while Abendsoone and Teddy bear varieties recorded (50%) the similarity matrix; more variability than varieties before.

The polymorphism of untreated varieties were (76.92%), while for untreated with treated varieties with 100 mM and 250 mM were (65%); Changes in gene expression and an enhanced risk of protein damage induce profound alterations in DNA remodelling, transcription and protein metabolism-protein biosynthesis as well as protein degradation¹⁸.

Varietal differences were verified by the presence or absence and the thickness of a particular band, these variations may be due to the genetic diversity in addition to the environmental conditions. The change in the genetic groups in the plant cell so that the plant can adapt to environmental changes also may be due to factors within the plant as organizations. Or to the chemicals caused by metabolism affect the activity of genes^{29,28}.

The effect of salt were positive suggested by appearance of bands in varieties treated not founded in controls (two bands in 250 mM before and after 18.5 kDa in Abendsoone variety) Figure (4); These bands may be linked with *Helianthus annuus* tolerance, also may be explained on the base of mutational event at the regulatory system of unexpected gene (s) that activated it², in plants, cold, drought, and salt stresses all stimulate the accumulation of compatible osmolytes and antioxidants¹². In yeast and in animals, mitogenactivated protein kinase (MAPK) pathways are responsible for the production of compatible osmolytes and antioxidants. These MAPK pathways are activated by receptors/ sensors such as protein tyrosine kinases, G-protein-coupled receptors, and two-component histidine kinases. Among these receptor-type proteins, histidine kinases have been unambiguously identified in plants. An Arabidopsis histidine kinase, AtHK1, can complement mutations in the yeast two-component histidine kinase sensor SLN1, and therefore may be involved in osmotic stress signal transduction in plants³². New polypeptides should be stress proteins. this plant adapts to sodium chloride salinizations by synthesizing stress proteins. The appearance and disappearance of bands under NaCl stress is for adaptation under saline conditions. However, disappearance of bands in treated varieties Figure (4); suggests that gene expression is altered under NaCl saline conditions²⁶.

Figure (3): Representative 2-DE gel illustrating the resolution of Teddy bear variety proteins extracted from leaves. (The arrows: spots, A: untreated variety, B: treated variety with 250 mM, ↑:Exist, ↑:New appearance)

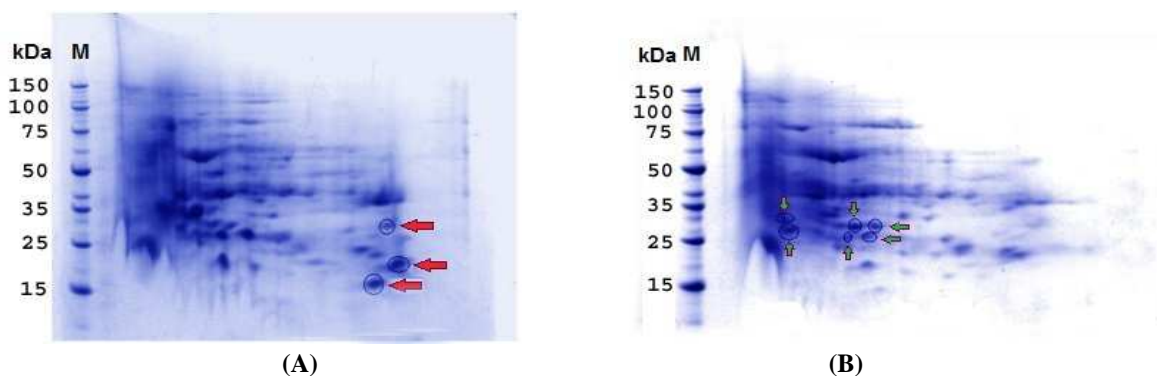


Figure (4): SDS-PAGE separation of proteins extracted from sunflower leaves treated with different concentrations of NaCl. (C untreated control, 100 mM and 250 mM, HMW: High molecular weight ,LMW: Low molecular weight). Line 2 and 3 (Variety 1): disappearance of bands between 62-95 kDa. Variety 3: between 36-60 kDa and between 67-220 kDa. Variety 4: before 14.3 kDa, between 20.1-29 kDa, at 43 kDa and at 97.4 kDa, ↑:New appearance .

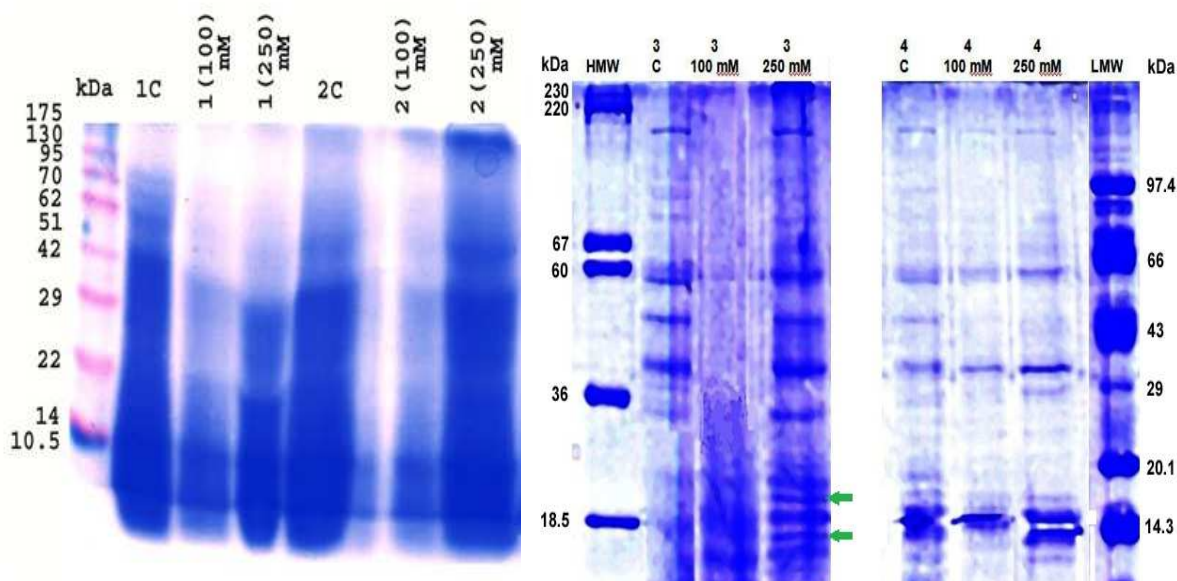
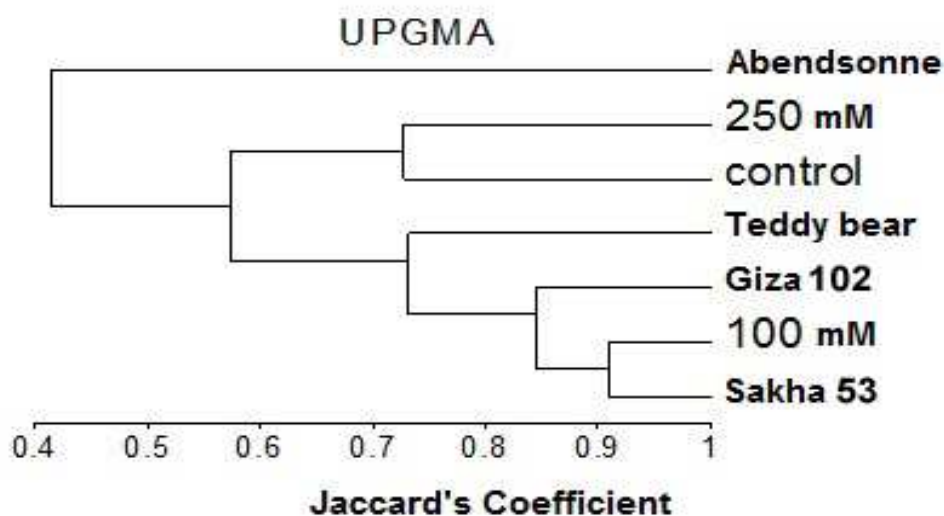


Table (4):Total similarity matrix

Similarity matrix	Sakha 53	Giza 102	Abendsoone	Teddy bear	Control	100 mM	250 mM
Sakha 53	1						
Giza 102	0.8	1					
Abendsoone	0.5	0.667	1				
Teddy bear	0.667	0.8	0.5	1			
Control	0.727	0.444	0	0.545	1		
100 mM	0.909	0.889	0.571	0.727	0.6	1	
250 mM	0.667	0.4	0.25	0.667	0.727	0.545	1
	Sakha 53	Giza 102	Abendsoone	Teddy bear	Control	100 mM	250 mM

Figure (5):Total protein dendrogram



CONCLUSION

In light of the physiological results the effect of salt on percentage of germination and root length was significant and highly significant decreased with application of salt treatment with 50 mM, 100 mM, 150 mM, 200 mM and 250 mM in most of varieties (Sakha 53, Giza 102, Abendsoone, Teddy bear, Gigante and white Hassawi). Biochemical analysis results showed that the proteomics analysis on Teddy bear variety by 2D gel electrophoresis, showed the effect of salt with appearance of spots in variety treated with 250 mM not founded in the control similar disappearance of spots founded in the control and not in the sample treated. Proteomic analysis confined only to Teddy bear variety, because of difficulty of applying it in most of the research laboratories and do not let the rest of the application varieties, therefore the application was limited on one variety. The application on the rest of varieties are considered future studies, because it was the latest techniques used to relationship between protein and their gene expression. The SDS-PAGE showed (76.92%) of polymorphic between untreated varieties (Sakha 53, Giza 102, Abendsoone and Teddy bear), while the effect of sodium chloride were significant suggested with appearance of bands not detected in control and disappearance of bands from treated varieties and with cluster analysis. Finally from the importance to continue of proteomic application on other varieties of sunflower. Proteomic application is a useful technique to study the genotype (the gene diversity) for the effect of different factors of stress as well as salts on plants varieties with determination of the nature of stress proteins and its contribution in the salt tolerance mechanism of sunflower.

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