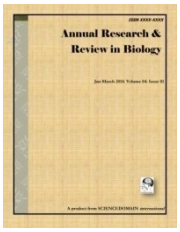

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Comparison of Protein Changes in the Leaves of Two Bread Wheat Cultivars with Different Sensitivity under Salt Stress

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Wheat performance under both Irrigated and dry land areas is affected by salinity and decreased. Like other plants, wheat cultivars try to indicate salt resistance through biochemical, physiological and morphological changes. In this study, responsive proteins in wheat leaves under salt stress were investigated by proteomics technique.
Study Design: Factorial experiment based on a randomized complete block design
Methodology: In this research, tolerant (Roshan) and sensitive (Ghods) cultivars were treated by 200mM salinity during 17 days. A proteomic technique was used to study proteins of leaf. Proteins of the leaves were extracted by TCA–acetone, and separated by two-dimensional gel electrophoresis at pH 4–7.
Results: 200 repeatable spots were identified by using Melanie software and analyzed statically. 24 and 12 responsive spots were identified by using MALDI TOF-TOF mass spectrometry in tolerant and sensitive cultivars respectively. 5 responsive proteins were overlap between both cultivars. In tolerant cultivar, Expression of 19 spots was increased and expression of 5 spots was decreased. All the identified proteins are involved in the regulation of carbohydrate, energy and nitrogen metabolism, photosynthesis related

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proteins, ROS scavenging proteins, signaling related protein, transporters and chaperon. But, all identified proteins except one of them in sensitive cultivar were down regulated under salt stress.
Conclusion: Based on this study, salt tolerant cultivar could alleviate salt stress. But salt sensitive cultivar couldn't tolerate salinity and it seems that salt stress have destructive effects on its proteins. In other words, protein compositions of salt sensitive cultivar are more deeply damaged by salt stress than salt tolerant cultivar and protein decay could be a marker of the sensitivity of the concerned cultivar.

Keywords: Bread wheat; proteomics and salt stress.

1. INTRODUCTION

Salt stress is an important abiotic stress affecting crops. High salinity of soil irreversibly affects growth of crops [1]. This stress can reduce yields of crops up to 50% [2]. Wheat is an important crop in the world affecting by salinity in irrigated and dry land agriculture [3]. Plant response to salinity occur in two steps: in first step, osmotic stress cause growth reduction whereas in second step, ionic stress cause ionic toxification and nutrient deficient that leads molecular degradation, growth suspension and plant death [3].

Plants try to indicate salt tolerance through biochemical, physiological and morphological changes; these changes are resulting from changes of gene expression that encode special proteins [4]. Tolerance to salt stress is associated with changes of protein levels. Salt stress leads to reducing or increasing of protein expression or disappearing of some proteins [5]. Special changes in protein profiles has been showed in many plants under salinity and by the same way a lot of proteins that have prominent role in response to salinity, determined [6,7].

Based on previous study on bread and durum wheat and by using proteomics technique, it is determined that ROS scavenging proteins were up regulated under salt stress [6,7,8]. ABC transporter and HSP70 were up regulated under salt stress in bread wheat [7]. Claes et al. [9] indicated that salt stress cause accumulation of a lectin like protein in rice. ABA responsive proteins such as LEA proteins accumulated in high levels in tolerant rice cultivars than sensitive cultivars [10,11]. ABA and jasmonates antagonistically regulate salt induced proteins. Six salt induced proteins (peroxidase, SaltT, pathogenesis related (PR) protein 10, PR1 and two unknown proteins) after jasmonate treatment and two proteins after ABA treatment (OSR40C1 and group 3 LEA) accumulated [11]. Ndimba et al. [12] identified 75 responsive proteins by using proteomics under salinity and these proteins are involved in H^+ transporting ATP ases, signal transduction related proteins, transcription/translation related proteins, detoxifying enzymes, amino acid and purine biosynthesis related proteins, proteolytic enzymes, heat shock proteins, carbohydrate metabolism associated proteins and proteins with no known biological functions.

With accumulation of the vast volume of genomic sequences in databases, researchers concluded that complete genomic sequences is not enough for describing biological functions. Indeed proteomics can be a complementary of genomics because of its focus on gene production that are active factors in the cell [13].

Studies showed that proteins frequency hasn't correlation with mRNA levels all the time because of posttranslational modifications [14]. Furthermore, only study of proteins itself can provide information about real volume and its activities [15].

It needs to increase tolerance of plants to salt stress [16]. Understanding of mechanisms that plants used under stress can help plant breeder to improved tolerance of plants. Our purpose in this research is investigation of changes in protein profiles in two sensitive and tolerance cultivars under salt stress and comparison of response of these cultivars under salt stress.

2. MATERIAL AND METHOD

2.1 Plant Material and Salinity Treatment

Wheat seeds of a salt-tolerant (Roshan) and salt sensitive (Ghods) cultivars [17] were used to study the response of two cultivars with different sensitivity to long-term salt stress. Seeds were surface-sterilized with 1% hypochlorite and planted into pots (25 cm in diameter), containing a mixture of perlite and cocopit (3:1 by volume) using a factorial experiment based on a randomized complete block design with three replications. The number of seeds planted in each pot was eight. Initially pots were irrigated with tap water for a week, then with a quarter strength Hoagland's solution for one week more and then with half strength Hoagland's solution. Twenty days after sowing, the pots were moved to a growth chamber to satisfy the vernalization requirement by exposing the plants to a temperature of 2-4ºC for 4 weeks. After 4 weeks the pots were placed back in the glasshouse and thinned to five plants per pot. At the 4-leaf stage, plants were exposed to 0 (control) or 200 mM of NaCl. NaCl (25 mM) was added twice in the first and second day and NaCl (50 mM) was added twice at third day (at 7:00 am and 5:00 pm) to a final concentration of 200 mM and supplementary $CaCl_2$ was also added to give a final concentration of 12 mM, because of Ca^{2+} deficiency which caused by high concentration of sodium (Na^+). Salt treatment was maintained for 17 d. After 17 days, newly matured leaf from wheat plants was harvested by cutting at the base of leaf, then quickly wrapped in aluminum foil pouch and immediately frozen in liquid nitrogen and stored at -80ºC.

2.2 Na^+ and K^+ Measurement

Blades of harvested leafs were washed in distilled water, oven dried at 70ºC for 48 h and grounded after being weighed. Na^+ and K^+ concentration was taken from the chloride acid (2 N) extract of the samples that had been burned at 580ºC for 4h, using a flame photometer.

2.3 Statistical Analysis

Statistical analysis was carried out using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and the t-test was used to evaluate differences between mean values.

2.4 Protein Extraction

Harvested leaves were ground in a mortar containing liquid nitrogen until a fine powder was obtained, and then the proteins were extracted following the procedure described by Damerval et al. [18] with some modifications. Briefly, 1 g of powder was suspended in an

ice-cold TCA Extraction Solution (10% w/v trichloroacetic acid (TCA) in acetone with 0.07% w/v DTT) for at least 1 h at -20ºC and centrifuged for 20 min at 35000×g. The pellets were rinsed twice with Sample Washing Solution (acetone containing 0.07% w/v DTT) for 1 h at -20ºC and then lyophilized. The resulting pellet was solubilized in lysis buffer (9 M urea, 4% CHAPS, 35 mM Tris base, 1% w/v DTT, and 1% v/v Ampholyte pH 3-10) for 1 h at room temperature and then centrifuged at 12000×g for 15 min. The supernatant was carefully collected and aliquoted and stored at -80ºC until 2-DE. The protein concentrations were quantified according to the Bradford method [19] using BSA as standard.

2.5 Two-Dimensional Gel Electrophoresis (2-DE)

2-DE was performed according to Görg et al. [20]. The IPG strips (pH 4–7,17cm length, Bio-Rad) were rehydrated at RT for 12-16 hin 320 µL rehydration solution (8 M w/v urea, 2% w/v CHAPS, 20mM DTT, 2% v/v IPG buffers (pH 3-10) and 0.002% Bromophenol blue) in a re-swelling tray (Amersham Pharmacia Biotech, Uppsala, Sweden).For analytical and preparative gels, 120 µg and 1.5 mg protein were loaded, respectively. Isoelectric focusing was performed at 20ºC with Multiphor II and a Dry Strip Kit (Amersham Pharmacia Biotech). The running conditions were as follows: 150 Vh at 0-300 V, 300 Vh at 300-500 V, followed by 2,000 Vh at 500-3,500 V and finally 39,500 Vh at 3,500 V. Focused strips were equilibrated for 15 min in 8 ml equilibration solution (50 mM Tris-HCl, pH 8.8, 6 M w/v urea, 30% w/v glycerol, 2% w/v SDS, 1% w/v DTT and 0.002% bromophenol blue).

The second dimension was performed on a 12.5% SDS-polyacrylamide gel using a Protean II Xi Cell (Bio-Rad).The protein spots in analytical gels were visualized by silver nitrate according to Bloom's protocol [21]. Preparative gels were stained with colloidal CBB G-250. Each treatment was replicated thrice.

2.6 Image Acquisition and Data Analysis

The silver-stained 2-D gels were scanned using a GS800 Calibrated densitometer (Bio-Rad) in transmissive mode. Spot detection, quantification, and matching were performed using Melanie 6.02 software (Genebio, Geneva, Switzerland). Briefly, the gels were analyzed in automated mode, followed by manual spot editing, e.g., spot splitting or merging and verifying matches. The molecular masses of proteins on gels were determined by co-electrophoresis of standard protein markers (Amersham Pharmacia Biotech) and *p*I values of the proteins were determined by migration of the protein spots on 17 cm IPG (pH 4–7, linear) strips.

The volume of each spot from three replicate gels was normalized against total spot volume, quantified, and subjected to a t-test ($p \leq 0.01$). Only those spots that were present on all three replicate gels were quantified.

2.7 Protein identification and Database Search

Spots were manually excised from preparative CBB stained gels, de-stained for 1 h at room temperature using a freshly prepared washing solution consisting of 100% acetonitrile/50 mM ammonium bicarbonate (NH_4CHO_3) (50:50 v/v). Washing solution was removed and spots were left to dry for 30 min at 37ºC. Proteins were digested using a trypsin solution containing 12ng μL^{-1} (10 µL) trypsin in 50 mM ammonium bicarbonate solution. This reaction was left to proceed for 45 min at 4ºC. Excess of tryps in solution was removed and 20 µL of

50mM ammonium bicarbonate was added before gel pieces were placed in a 37ºC incubator overnight.

A 1 µL aliquot of each fraction was applied directly to the ground steel MALDI target plate, followed immediately by an equal volume of a freshly-prepared 5 mg mL^{-1} solution of 4-hydroxy-α-cyano-cinnamic acid (Sigma) in 50% aqueous (v:v) acetonitrile containing 0.1% trifluoroacetic acid (v:v).

Positive-ion MALDI mass spectra were obtained using a Bruker ultraflex III in reflectron mode, equipped with a Nd: YAG smart beam laser. MS spectra were acquired over a mass range of m/z 800-4000. For each spot the ten strongest peaks of interest, with an S/N greater than 10, were selected for MS/MS fragmentation. Fragmentation was performed without the introduction of a collision gas. The default calibration was used for MS/MS spectra, which were baseline-subtracted and smoothed (Savitsky-Golay, width 0.15 m/z, cycles 4); monoisotopic peak detection used a SNAP averagine algorithm (C4.9384,N 1.3577,O1.4773,S 0.0417,H 7.7583) with a minimum S/N of 3. Bruker flex Analysis software was used to perform the spectral processing and peak list generation for both the MS and MS/MS spectra.

Combined mass spectral and tandem mass spectral data were submitted to database searching using a locally-running copy of the Mascot program (Matrix Science Ltd., version 2.1), through the Bruker BioTools interface (version 3.1). Search criteria included: Enzyme, Trypsin; Variable modifications, Oxidation (M); Peptide tolerance, 200ppm; MS/MS tolerance, 0.8 Da; Instrument, MALDI-TOF-TOF. The search criteria also included Carbamidomethyl (C) as a fixed modification for all alkylated samples. The database search was run against NCBI non-redundant protein database Viridiplantae (20080218; 5519594 sequences; 1911975371 residues).

3. RESULTS AND DISCUSSIONS

3.1 Ion Concentration in Leaf 4

Maintaining of high cytosolic K^+/Na^+ ratio is an important aspect of salt tolerance in crops [22]. K^+ is essential to all plant's life [22] because more than 50 enzymes are activated by K^+ and high concentration of K^+ is essential for protein synthesis. There are some physicochemical similarities between K^+ and Na^+, Na^+ can compete with K^+ for major binding sites in the key metabolic processes in cytoplasm such as enzymatic reactions, protein synthesis and ribosome functions [23,24]. Because Na^+ cannot play the role of K^+ [25], it can disrupt different enzymatic process and protein synthesis at high concentration [24].

In this study, K^+/Na^+ ratio in Roshan cultivar was higher than Ghods cultivar under salt stress Fig. 1. This indicated that Roshan cultivar could maintain proper concentration of K^+ comparing to Ghods cultivar. It seems in this condition Roshan cultivar can change the expression of salt induced gens to alleviate salt stress effects, but Na^+ concentration of Gods is elevated and probably cause destructive effects on enzymes and Protein synthesis that was shown in this study.

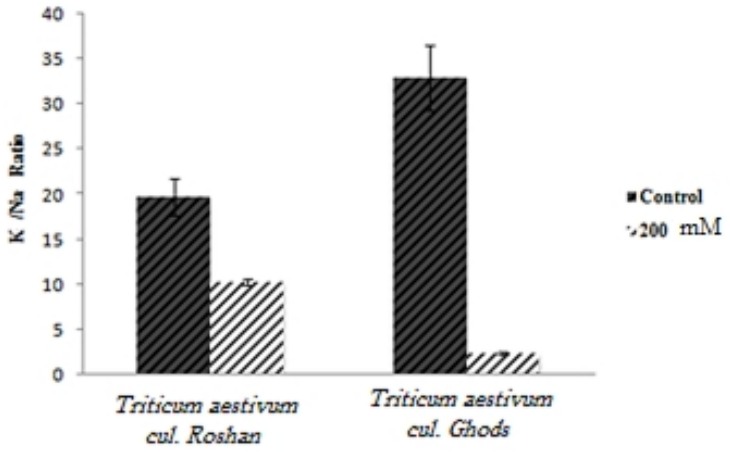

Fig. 1. Values of means and standard error of K^+/Na^+ ratio under untreated and treated conditions

3.2 Analysis of Two-dimensional Gel Electrophoresis

After analyzing all gels by Melanie software more than 200 responsive spot were identified Fig. 2. 24 responsive spots of Roshan cultivar were identified by using MALDI-TOF-TOF. Of these, 19 spots were up-regulated, and 5 were down-expressed. 12 responsive spot of Ghods cultivar were identified by using MALDI-TOF-TOF too. Of these, 1 resulted up-regulated, and 11 were down-regulated. Eight indentified proteins were common between Roshan and Ghods cultivars (ribulose-1,5-bisphosphate carboxylase activase, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, chloroplastic triosephosphate isomerase, chloroplastic fructose-bisphosphate aldolase, mitochondrial ATP synthase precursor, plastid glutamine synthetase, chloroplastic 2-cys peroxiredoxin BAS1, and 70 kDa heat shock protein).

Results indicated that in both cultivars some spots identified as one protein. For example, Rubisco small subunit identified in three spot (spots 18, 129 and 130), 2-cys peroxiredoxin identified in three spot (spots 57,61 and 97), oxygen evolving enhancer protein 2 identified in three spot (spots 34,36 and 193), Rubisco activase identified in three spot (spots 150,151 and 153) and auxin-binding protein ABP20 precursor identified in two spots (spots 15 and 16). These types of proteins were identified already [6,7,8]. The reason of this phenomenon probably are some important feature of proteins like presence of isoforms of proteins, post translational modifications, translation from alternative spliced mRNA and protein degradation. Post translational modifications such as glycosylation and phosphorylation can change molecular weight and isoelectric point [8]. Spots 97 and 36 had most and least induction factor (IF) in Roshan cultivar. Spot 49 had least IF in Ghods cultivar.

3.3 Identified Proteins of Roshan Cultivar

All the identified proteins are involved in the regulation of carbohydrate, energy and nitrogen metabolism, photosynthesis related proteins, ROS scavenging proteins, signaling related protein, transporters and chaperon.

Photosynthetic related proteins include Rubisco activase (spots 150,151 and 153), Rubisco large subunit (spot 169), Rubisco small subunit (spots 18,129 and 130), chloroplastic triosephosphate isomerase (spot 47), Chloroplastic fructose-bisphosphate aldolase (spot 112), Oxygen evolving enhancer protein 2 (spots 34,36 and 193). ROS scavenging proteins consist of ascorbate peroxidase (spot 49), Cu/Zn superoxide dismutase (spot 65) and 2-cys peroxiredoxin (spots 57, 61 and 97). Proteins involved in carbohydrate metabolism comprise of cytosolic malate dehydrogenase (spot 131) and phosphoglycerate mutase (spot 26). Nitrogen and energy metabolism, chaperon, transporters and signaling related protein are represented by plastid glutamine synthetase (spot 158), mitochondrial ATP synthase precursor (spot 124), 70 kDa heat shock protein (spot 172), ABC transporter (spot 48) and BRI1-KD interacting protein 114 (spot 19) respectively Table 1.

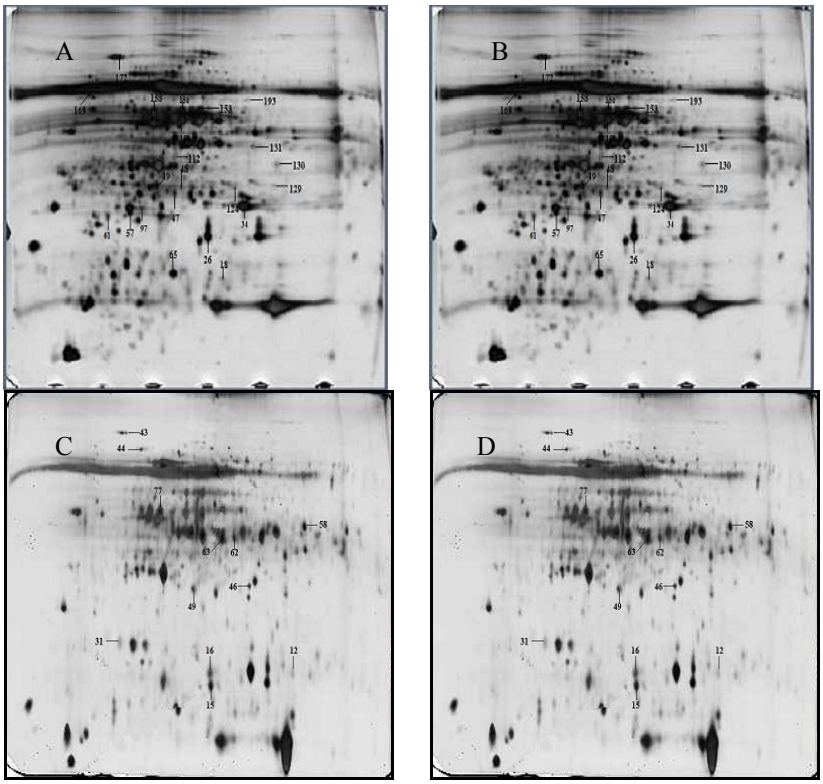

Fig. 2. 2-D Page protein profile of leaf 5. Protein (120 mg) was loaded on 18 cm IPG strip with a linear gradient (pH 4–7) and SDS-PAGE was performed with a 12% gel. Proteins were visualized by silver staining. The identified protein spots are marked. (A) Roshan, control. (B) Roshan, salinity. (C) Ghods, salinity. (D) Ghods, control

As mentioned above, In Roshan cultivar, 12 proteins were involved in photosynthesis. Of these, expression of Rubisco activase (spots 150,151 and 153), Rubisco large subunit (spot 169), Rubisco small subunit (spots 18,129 and130), chloroplastic triosephosphate isomerase (spot 47) increased and others decreased under salt stress. Rubisco is the key enzyme of Calvin cycle [26]. Environmental stress can reversibly or irreversibly inactivate Rubisco. Inactivated Rubisco probably is replaced by new synthesized enzymes. The main role of Rubisco activase is maintaining of catalytic function of Rubisco by remove of sugar phosphates from active site of carbamylated and uncarbamylated Rubisco [27]. Salt stress

lower CO_2 levels in stroma and in this condition Rubisco is inactivated through binding of suppressive sugars. Up regulated of activase can activate Rubisco under low CO_2 levels [28]. Increasing.

of chloroplastic triosephosphate isomerase probably can provide sufficient energy for ROS scavenging and recovery of injuries resulted from oxidative degradation. Some reports indicated increasing of triosephosphate isomerase expression in leaves of bread wheat [6, 7], durum wheat [8] and rice [29] under salinity. Decreasing of Oxygen evolving enhancer protein 2 (OEE2) can lower the level of oxygen releasing leading to reduction of ROS production therefore photosystem II can be saved from more degradation. Down regulated of OEE2 has been indicated in durum wheat [8] and potato [30] under salinity stress. Chloroplastic fructose-bisphosphate aldolase is involved in Calvin cycle and starch biosynthesis by production of fructose 1,6-bisphosphate from combination of D-glyceraldehyde 3-phosphate and D-hydroxy acetone phosphate [31]. Down regulated of this enzyme probably can lead to accumulation of energy in chloroplast.

Cytosolic malate dehydrogenase and phosphoglycerate mutase belong to the carbohydrate metabolism and upregulated under salt stress. Expression increscent of these enzymes probably can produce more energy for defense processes. Inducement of Cytosolic malate dehydrogenase observed in leaf of bread wheat [7], Arabidopsis [12], *Thellungiella halophila* [32] and barley and maize [33]. Induce of phosphoglycerate mutase observed in durum wheat [8] under salt and rice [34] under cold stress.

Five identified protein of Roshan cultivar (ascorbate peroxidase (spot 49), Cu/Zn superoxide dismutase (spot 65) and 2-cys peroxiredoxin (spots 57, 61 and 97)), belong to the ROS scavenging proteins. All of these proteins are up regulated under salinity. ROSs can degrade cell component and act as signaling molecules [35]. Roshan cultivar probably regulates ROS levels through complex mechanisms of the mentioned enzymes that their functions are ROS scavenging and cell defense. According to previous reports, expression of ascorbate peroxidase increased in bread [7] and durum [8] wheat and rice [36] under salt stress. Several reports mentioned upregulation of Cu/Zn superoxide dismutase under salinity [8,12,37,38].

Plastid glutamine synthetase is involved in nitrogen metabolism. This protein was upregulated in Roshan cultivar under salinity. This protein can provide nitrogen groups for biosynthesis of all nitrogen components of plants [39]. In some studies, increasing of glutamine synthetase expression has been indicated in leaves of bread [7] and durum [8] wheat, rice [34] and root of fox tail millet [40].

Mitochondrial ATP synthase precursor was down regulated under salt stress in Roshan cultivar. ATP synthase enzyme is an important enzyme for maintain of ion homeostasis in plant cells. Reduction in its expression probably is related with reduction of photosynthesis rate under salinity. This result is similar with results of Carosu et al. [8]. 70 kDa heat shock protein (HSP70) was up regulated under salt stress in Roshan cultivar. HSP70 is involved in correct folding of protein and protection of them against denaturing and aggregation [12]. Inducement of this protein has been observed in several studies [7,12,41]. ABC transporter was upregulated under salt stress in Roshan cultivar. This protein has important role in transportation of secondary metabolite such as alkaloids, terpenoids, polyphenols and quinine that play important role in defense and tolerance to abiotic stress [7]. Expression of BRI1-KD interacting protein 114 was increased under salt stress in Roshan cultivar. BRI1-KD interacting protein 114 is a type of Nucleoside Diphosphate Protein Kinase (NDPK).

NDPK in presence of Mg^{2+} catalyze transfer of phosphate groups between nucleoside phosphates [42]. Signal transfer system by BRI1 is like to the G protein signal transfer system and probably the identified protein interfere in supplying nucleoside three phosphate needed for BRI1 signal transfer system. Katz et al. [42] reported increasing of NDPK expression in salt tolerance alga, *Dunaliella salina*.

3.4 Identified Proteins of Ghods Cultivar

All of identified proteins are involved in the regulation of Energy and nitrogen metabolism, Photosynthesis related proteins, Signaling related protein**,** ROS scavenging proteins and Chaperon.

Photosynthetic related proteins include Rubisco activase (spot 58), Rubisco large subunit (spot 12), chloroplastic triosephosphate isomerase (spot 49), Chloroplastic fructose-bisphosphate aldolase (spot 63) and RuBisCO large subunit-binding protein subunit (spot 44). Proteins involved in Nitrogen metabolism comprise of plastid glutamine synthetase (spot 77) and Cysteine synthase (spot 62). Signaling related proteins include auxin-binding protein ABP20 precursor (spots 15 and 16). Proteins involved in energy metabolism, chaperon and ROS scavenging are represented by Mitochondrial ATP synthase precursor (spot 46), 70 kDa heat shock protein (spot 43), 2-cys peroxiredoxin (spot 31) respectively Table 2.

Of twelve identified proteins, five spots are involved in photosynthesis, two spots are involved in Signaling, one spot is involved in energy metabolism, two spots are involved in nitrogen metabolism, one spot is involved in ROS scavenging and one spot belong to the chaperon. The main point is that all the identified proteins except one protein (plastid glutamine synthetase) were down regulated. On the other hand, the most proteins in sensitive cultivar are deeply destroyed by salt stress. This phenomenon can be a marker for identifying of salt sensitive cultivars. These results are similar with the results of Oureghi et al. [43]. In their experiment, two cultivars of durum and bread wheat with different sensitivity were affected to salt stress. Their results indicated that of 12 responsive proteins of salt sensitive cultivar, 11 responsive spots were down regulated. They concluded that salt stress promote a destructive effect on the polypeptidic composition of the salt sensitive cultivar. Moreover, they mentioned that the evaluation of the protein decay due to salt stress could be a marker of the sensitivity of the concerned cultivar or species toward NaCl [43].

Table 1. Identified Salt Responsive Proteins Using MALDI TOF-TOF in Roshan cultivar

Identity	Spot ID	[a]gi|Number	[b]Expression change	% [c]Coverage	[d]MS Score	[e]The pI/MW(kDa)	[f]Exp pI/MW(kDa)
Photosynthesis related proteins							
ribulose 1,5-bisphosphate carboxylase activase isoform 1 [*Hordeum vulgare subsp. vulgare*]	153	gi|167096	+	31	331	8.62 ، 47.34	5.53 ، 49
ribulose 1,5-bisphosphate carboxylase activase isoform 1 [*Hordeum vulgare subsp. vulgare*]	150	gi|167096	+	49	537	8.62 ، 47.34	5.32, 45
chloroplast ribulose-1,5-bisphosphate carboxylase activase [*Triticum aestivum*]	151	gi|115392208	+	39	361	6.52, 40.26	5.31, 49
Oxygen-evolving enhancer protein 2, chloroplastic	36	gi|131394	-	48	368	8.84 ، 27.42	5.58 ، 26
Oxygen-evolving enhancer protein 2, chloroplastic	34	gi|131394	-	67	552	8.84 ، 27.42	5.94, 25
Oxygen-evolving enhancer protein 2, chloroplastic	193	gi|131394	-	33	196	8.84 ، 27.42	5.96, 52
ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit [*Distichia acicularis*]	169	gi|21684943	+	33	353	6.2 ، 48.65	4.34 ، 54
ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit [*Triticum aestivum*]	130	gi|4038719	+	56	255	8.83، 18.80	6.19 ، 32
Ribulose bisphosphate carboxylase small chain clone 512	18	gi|132107	+	36	60	5.8 ، 13.27	5.75 ، 16
putative rubisco small subunit [*Triticum turgidum subsp. durum*]	129	gi|62176930	+	52	227	8.59 ، 19.32	6.17 ، 29
Triosephosphate isomerase, chloroplastic	47	gi|1174745	+	28	100	6 ، 31.95	5.23 ، 27
chloroplast fructose-bisphosphate aldolase [*Triticum aestivum*]	112	gi|223018643	-	21	345	5.9 ،42.21	5.22 ، 34
Carbohydrate metabolism							
cytosolic malate dehydrogenase [*Triticum aestivum*]	131	gi|49343245	+	41	115	5.7 ، 35.80	6.004 ، 37
phosphoglycerate mutase [*Triticum aestivum*]	26	gi|32400802	+	66	454	5.3 ، 29.61	5.61 ، 21
Energy metabolism							
mitochondrial ATP synthase precursor [*Triticum aestivum*]	124	gi|47607439	-	37	164	8.8 ، 27.09	5.85 ، 28
Nitrogen metabolism							
plastid glutamine synthetase isoform GS2b [*Triticum aestivum*	158	gi|71362638	+	36	541	6 ، 46.95	4.98 ، 48
ROS scavenging proteins							
ascorbate peroxidase [*Hordeum vulgare*]	49	gi|15808779	+	44	385	5 ، 27.96	5.02 ، 29
Cu/Zn superoxide dismutase [*Triticum aestivum*]	65	gi|1572627	+	43	437	5.3 ، 20.35	5.21 ،17
2-Cys peroxiredoxin BAS1, chloroplastic	57	gi|2499477	+	54	708	5.4 ، 23.39	4.65 ، 25
2-Cys peroxiredoxin BAS1, chloroplastic	97	gi|2499477	+	30	92	5.4 ، 23.39	4.81, 25
2-Cys peroxiredoxin BAS1, chloroplastic	61	gi|2499477	+	54	359	5.4 ، 23.39	4.42, 24

Table 1 continued……..

Chaperon							
70 kDa heat shock protein [*Triticum aestivum*	172	gi|254211611	+	35	578	4.9 ‹ 73.72	4.53 ‹ 60
Transporters							
abc transporter, putative [*Ricinus communis*]	48	gi|255565291	+	9	69	6.4 ‹36.00	5.3 ‹ 28
Signaling related protein							
BRI1-KD interacting protein 114 [*Oryza sativa* Japonica Group]	19	gi|42733490	+	42	288	5.7, 16.703	5.8, 15

a. *Accession number in NCBI, SWISS Prot*

b. Expression changes of proteins under salt treatment than control conditions in Roshan cultivar. + and – indicate protein spots whose abundance increase (+) or decrease (–)

c. percentage of predicated protein sequence covered by matched sequences.

d. statistical probability of true positive identification of the predicted protein calculated by MASCOT

e. Tpl/ Tmw: pI of predicted protein /molecular mass of predicted protein.

Epl /Emw: pI of protein on gel /molecular mass of protein on gel.

Table 2. Identified Salt Responsive Proteins Using MALDI TOF-TOF in Ghods cultivar

Identity	Spot ID	[a]gi|Number	[b]Expression change	% [c]Coverage	[d]MS Score	[e]The pI/MW(kDa)	[f]Exp pI/MW(kDa)
Photosynthesis related proteins							
ribulose-1,5-bisphosphate carboxylase activase [*Triticum aestivum*]	58	gi|37783283	-	66	251	4.8, 22.49	6.33, 41
ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	12	gi|222543970	-	27	208	5.7, 23.31	6.24, 19
Triosephosphate isomerase, chloroplastic;	49	gi|1174745	-	24	100	6, 31.95	5.38, 30
chloroplast fructose-bisphosphate aldolase [*Triticum aestivum*]	63	gi|223018643	-	32	345	5.9, 42.21	5.71, 38
RuBisCO large subunit-binding protein subunit	44	gi|134102	-	60	632	4.7, 57.65	4.89, 54
Signaling related protein							
auxin-binding protein ABP20 precursor [*Zea mays*]	15	gi|195616892	-	11	87	6, 20.55	5.58, 18
auxin-binding protein ABP20 precursor [*Zea mays*]	16	gi|195616892	-	16	103	6, 20.55	5.57, 20
Energy metabolism							
mitochondrial ATP synthase precursor [*Triticum aestivum*]	46	gi|47607439	-	58	310	8.8, 27.09	5.91, 30
Nitrogen metabolism							
plastid glutamine synthetase isoform GS2c [*Triticum aestivum*]	77	gi|71362640	+	29	488	5.7, 47.01	5.02, 45
Cysteine synthase; AltName: Full=O-acetylserin	62	gi|585032	-	32	218	5.3, 34.20	5.8, 39
ROS scavenging proteins							
2-Cys peroxiredoxin BAS1, chloroplastic	31	gi|2499477	-	40	158	5.4, 23.39	4.75, 23
Chaperon							
70 kDa heat shock protein [*Triticum aestivum*]	43	gi|254211611	-	35	554	4.9, 73.72	4.79, 54

4. CONCLUSION

We investigated protein changes of two salt sensitive and tolerance cultivars by using proteomics. Tolerance cultivar, Roshan, could alleviate effects of salt stress by changing of protein expression involved in photosynthesis, carbohydrate, energy and nitrogen metabolism, ROS scavenging protein, chaperon, ABC transporter and BRI1-KD interacting protein 114. It means that salt stress wasn't severe for salt tolerant cultivar (Roshan) and this cultivar could alleviate salt stress. But salt sensitive cultivar (Ghods) couldn't tolerate salinity and it seems that salt stress have destructive effects on Gods' proteins. In other words, protein compositions of Ghods are more deeply destroyed by salt stress than Roshan cultivar. Based on this study, Protein decay could be a marker of the sensitivity of the cultivar.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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