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Proteome analysis of wheat *(Triticum aestivum* L.*)* root under sodium chloride stress

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Key words: NaCl stress, root proteome, two-dimensional electrophoresis, wheat.

Abstract

Salinity is the most important abiotic stresses adversely affect the quality and quantity of crops, in which 20% of the world's irrigated agricultural lands are affected by salinity. Wheat is an oldest and the first crop used for bread making for human nutrition. To investigate the response of wheat root to NaCl stress, a susceptible, Arta, and resistance, Bam, wheat varieties were grown under both non-stress and stress conditions. Stress plants were exposed to 250 mM of NaCl based on a completely randomized design with four replications. Proteins were extracted from root and proteomic analysis was performed by two-dimensional polyacrylamide gel electrophoresis. Proteome profile of root in susceptible variety lead to the identification of 21 differentially changed protein spots compare with 49 spots of tolerant variety. The regulatory changed proteins belongs to stress-responsive proteins and others related processes including metabolism and energy; scavenging of reactive oxygen species and detoxifying; protein translation, processing and degradation; cell wall-related proteins; Amino acid metabolism and hormone-related proteins; signal transduction network; cytoskeleton; transcription-related proteins.

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Introduction

Salinity is one of the most important abiotic stresses that adversely affect the quality and quantity of crops, so that 20% of the world's irrigated agricultural lands are affected by salinity (Zhao *etal*., 2007). Biological and abiotic stresses such as salinity, before any change in cytoplasmic calcium concentration and pH occur, it must somehow be understood by the plant. Salinity stress by osmotic stress and ion toxicity (sodium and chloride) and these tensions can be understood in both the inner and outer plasma membrane by transmembrane proteins or enzymes inside cytosolunderstood. Many osmotic raised in understanding drought stress sensors are activated by intense passion (Abdul Kader and Lindberg, 2010).

Salt interferes with plant growth and can lead to physiological drought and ionic toxicity. Thus, salinity and drought stress often affects the physiological aspects of plant metabolism, creating tension (Hyperionic and Hyper osmotic), and eventually plant will death. Salinity and drought stress have overlap on physiology because having salt in soil decrease the amount of water on it and leading to reduced water absorption. Salinity stresses cause ion stress, potassium ion, sodium ion ratio are change. External sodium ions can have a negative effect on the absorption of calcium ions. Salinity resulted increase the concentration of sodium ions and chloride in cytosol, the outcome could be detrimental to the cells. Sodium ion can eliminate membrane potential, thus facilitated the absorption of chloride. The high concentration of sodium ions (up to 100 mM), is toxicity for the cell metabolism and can prevent activity of many essential enzymes, cell division and expansion, causing membrane damage and osmotic imbalance and thus stop the growth. The high concentration of sodium ions can lead to the production of reactive oxygen species and reduction of photosynthesis. Potassium is an essential element required for growth. The concentration of potassium ions (due to severe salt stress) cause osmotic imbalance, problem in stomata functions and action of enzymes. Salinity damage cells in the leaf transpiration, resulting in prevention of growth and cause cell toxicity. Salts can accumulate in older leaves and cause death (Tuteja, 2007).

Several studies have used Proteomics to identify proteins in response to salinity. Many proteins have been found that match their words with the salt concentration is set. The proteins in the process of photosynthesis, photo respiration, transduction, metabolism, defense against oxidative stress, ion channels control the folding of proteins are involved (Joseph and Jini, 2010). The effect of salinity on plant growth is a two-step Changes in the osmotic effect of salt water, and toxic ions in salt increases the transpiration and leaf emerges. Changes in proteome of wheat, 30 days after exposure to 125 mM NaCl in the culture chamber were evaluated. A significant negative correlation between tolerance to salt and sodium concentration in wheat stems were observed. Protein expression and susceptible genotypes after 10 days of treatment with 125 mM sodium chloride, more than 5%, but the difference between the figures in different groups of protein modifications (over expression, knockdown, disappearance and appearance) from 1 to 8 % was variable (Saqib *et al*., 2006). Song (2007) to develop a better understanding of the roots of wheat, a map of soluble proteins in roots using a combination of MS/MS and MALDI TOF MS and 2-DE prepared. A total of 450 protein spots stained with silver in the pH range 4-7 View and identified 240 proteins were identified. These proteins are functionally divided into various groups compared with the wheat root proteome proteins involved in metabolism and transport were mentioned too. 25 proteins from 45 different proteins expressed in metabolism, energy production, growth and cell division, disease, and the secondary metabolites involved. They also showed that hybridization between two parental lines could be differences in protein expression in the offspring than their parents..

Proteomic analysis of leaves in different wheat cultivars to salinity stress on both the performance

and quality of gluten strength were investigated. Seedlings with four different salt concentrations (1, 5.1, 2 and 2.5%) were evaluated. Total protein was extracted from the leaves of plants under stress and non-stress conditions were separated by two-dimensional electrophoresis. Of 2358 protein spots, 125 spots showed significant changes in response to salinity. Using mass spectrometry, 52 spots were detected in response to salt in six functional groups. These groups include proteins associated with the transport, detoxification enzymes, ATP synthesis, carbon metabolism, formation of protein structure and protein with unknown biological function. Of these 52 spots, 26 spots up-regulated and 21 down-regulated spots and five spots showed several patterns of expression (Gao *et al.*, 2011).

Long-term effects of salt stress, and stress on plant seeds planted in the four-leaf stage, the levels were zero and 300 mM NaCl. Sampling by separating the fourth leaf of each plant was done 21 days after stress. The results of this experiment showed that among more than 500 protein spots repeatable, 124 spots were a significant difference between treatments. Proteins identified in the mechanism of photosynthesis, oxidative stress, translation, and protein were involved Trarsany message. The aim of the present work was to perform a comparative study by proteomics approach, based on two-dimensional poly-acrylamide gel electrophoresis (2D-PAGE) in order to identify salinity-related proteins in wheat *(Triticum aestivum)* varieties named Arta and Bam that differ in tolerance to the NaCl stress (Fatehi *et al.*, 2012).

Materials and methods

Plant growth and stress treatment

Plant material used in this study was two wheat *(Triricum aestivum* L.*)* cultivars; "Bam" and "Arta" known as tolerant and susceptible to NaCl stress, respectively. These seeds were taken from "Seed and Plant Improvement Institute", Karaj, IRAN. Experiment was conducted in completely randomized design with three replications. Treatments were combination of wheat, cultivars of wheat ("Arta" and "Bam") and two irrigation levels including normal and NaCl stress (250 mM). Stress was imposed by combination Nacl with water. Then roots were harvested and were immediately immersed in liquid nitrogen and stored at -80˚C until used for protein extraction.

Protein extraction

Total protein extracts were isolated from approximately 0.5 g of frozen leaf per biological replicate and suspended the fine powder in cold acetone containing 10% TCA and 0.07% 2-Mercapthoetanol. The resultant powder dissolved in lysis buffer containing 7 M Urea, 2 M thiourea, 2% CHAPS, 60 mM DDT and 1% ampholyte (pH:3-10). Protein concentration was determined by Bradford assay (Bradford, 1976).

Two-dimensional gel electrophoresis

IEF of proteins performed with 140 µg of protein extract using 11 cm tube gels and 3 mm diameter (O'Farrell, 1975). IEF gel solution consisted of 8 M urea, 3.5% polyacrylamide, and 2% NP-40, 2% ampholines (pH: 3.5-10.0 and pH: 5.0-8.0), ammonium persulfate and TEMED. The voltage settings of the IEF were a 200 V gradient for 30 min, a 400 V gradient for 16 h and a 600 V for 1h. Proteins in the tube gels separated on the basis of their IF. The second dimension SDS-PAGE gels, tube gels were subjected to the second dimension electrophoresis after transferring onto a 15% acrylamide separating gel and 5%b acrylamide stacking gel. After electrophoresis, we stained Proteins with Silver nitrate ($AgNO_3$).

Statistical analysis and spot identification

Gels were scanned using BioRad GS-800 scanner. Images analyses were performed with PDQuest™ software (BioRad). Selected spots were attributed to the corresponding proteins by search taking into account of their isoelectric point (pI) and molecular weight (MW) within databases especially "SWISS-2DPAGE" in EXPASY. After normalization, a one-way

ANOVA model was used to identify the differentially expressed protein spots between normal and stress. Protein identification was obtained from MSDB, NCBI and SwissProt protein database.

Result

Analysis of proteome

NaCl stress related proteins were investigated by 2D-PAGE and bioinformatics' databases in two *Triticum aestivum* cultivars: Bam (Salinity tolerant) and Arta (Salinity susceptible).

Table 1. Nacl responsive proteins identified in Bam cultivar. [a] Spot numbers given by PDQuest software/ [b] The component increased (↑), decreased (↓).

Spot no[a]	Identified protein	pI/MW experimental	pI/MW theoretical	Express. Level[b]	Accession no
1204	Iron deficiency-induced protein IDI1	4.1/23	5.23/23.46	↑	TC145151
1402	ACC oxidase (ACO2)	4.1/44	4.97/36.4	↓	At1g62380
1403	ACC oxidase (ACO2)	4.2/45	4.97/36.4	↓	At1g62380
2409	Late embryogenesis abundant protein	4.8/44	4.98/41.07	↑	TC139604
3113	Glutathione peroxidase	5.4/20	5.11/19.7	↑	SGN-U322657
3207	Probable nicotianamine synthase 7	5.3/35	5.1/35.24	↓	NP315772
3220	Aconitase C-terminal domain-containing	5.0/27	6.33/27.1	↑	At2g43090
3222	Nascent polypeptide-associated complex alpha chain	5.1/30	4.3/22.0	↑	At3g12390
3302	Late embryogenesis abundant protein	4.9/42	4.98/41.07	↑	TC139604
3303	Iron deficiency-induced protein IDI2	5.3/40	5.44/38.57	↓	TC147167
3503	Phosphopyruvate hydratase, enolase (LOS2)	5.1/50	5.54/48	↓	At2g36530
3506	Metalloendopeptidase	5.3/50	5.94/54.6	↓	At1g51980
4101	Methylamalonate semi-aldehyde dehydrogenase	5.4/21	7.54/58.01	↓	SGN-U316642
4202	Elongation factor1B alpha-subunit 2	5.4/35	4.57/24.58	↑	SGN-U313292
4203	Putative 6-phosphogluconolactonase	5.6/32	5.44/28.02	↓	SGN-U315096
4401	S-Adenosylmethionine synthetase 1	5.4/48	5.49/42.84	↓	TC131046
4605	60 kDa chaperonin subunit alpha	5.6/60	5.08/62.07	↑	SGN-U312542
4702	Transketolase-like protein	5.5/88	5.8/81.9	↓	At3g60750
4703	Protein disulphide isomerase (ATPDIL 1-2)	5.5/60	4.9/56.6	↑	At1g77510
4810	HSP90 protein	5.5/110	5.19/90.67	↑	SGN-U316401
5204	Peroxidase	5.8/35	5.91/36.55	↓	TC140370
5504	Aldehyde dehydrogenase (ALDH1a)	5.8/55	6.29/54.89	↓	SGN-U319484
5605	NADP quinine oxidoreductase	5.6/55	5.87/59.10	↑	SGN-U318400
5801	Serine protease	5.6/96	6.00/81.45	↓	SGN-U327796
6201	Putative nuclear RNA-binding protein A	6.1/34	6.37/40.42	↓	35_16328
6301	Actin	5.9/42	5.24/41.70	↓	SGN-U314753
6501	Ketol-acid reductoisomerase	5.9/55	6.50/63.35	↓	SGN-U312795
6701	P69C protein	5.9/55	5.27/70.68	↓	SGN-U313773
6709	Poly(A)-binding protein	6.1/73	6.60/70.82	↓	TC139323
7101	23 kDa jasmonate-induced protein	6.3/18	5.92/22.84	↑	TC138639
7303	Carboxymethylenebutenolidase-like protein	6.3/33	6.31/30.41	↑	TC139656
7401	Enolase (2-phosphoglycerate dehydratase)	6.2/47	5.68/47.80	↓	SGN-U312378
7402	ATP synthase F1 subunit 1	6.2/55	5.84/55.23	↓	SGN-U316882
7603	Dihydrolipoamide dehydrogenase precursor	6.3/60	6.90/52.81	↓	SGN-U314200
7604	Glycosyl hydrolase family 1 protein	6.4/60	6.95/60.3	↓	At3g09260
7709	Transketolase 1	6.5/90	6.16/80.11	↓	SGN-U312322
7711	Phosphogluconate dehydrogenase	6.4/63	7.02/53.58	↓	SGN-U319405
8104	(NAC) domain-containig protein	6.6/18	6.62/17.9	↑	At1g17880
8301	Glu dehydrogenase 2 (GDH2)	6.5/41	6.07/45.0	↓	At1g07440
8305	Fumarase (FUM1)/fumarate hydratase	6.6/42	8.01/53.5	↓	At2g47510
8308	Glyceraldehyde-3-phosphate dehydrogenase C subunit (GAPC)	6.7/40	6.62/37	↓	At3g04120
8402	Phosphogluconate dehydrogenase	6.6/48	7.02/53.9	↓	At3g02360
8405	Phosphogluconate dehydrogenase	6.7/48	7.02/53.9	↓	At3g02360
8504	Cytosolic 6-phosphogluconate dehydrogenase	6.6/53	6.58/51.58	↓	TC133105
8506	Glycosyl hydrolase family 1 protein	6.8/49	6.74/60.2	↓	At1g66280
8601	Glycosyl hydrolase family 1 protein	6.6/57	6.74/60.2	↓	At1g66280
8603	Glycosyl hydrolase family 1 protein	6.7/60	6.95/60.4	↓	At3g09260
8705	F23N19.10 stress-include protein	6.7/66.2	6.24/67.32	↓	TC139384
8706	F23N19.10 stress-include protein	6.8/66.2	6.24/67.32	↓	TC139384

Two comparison was conducted: first, Bam under normal and stress and second: between normal and stress condition that lead to reproducible detection of 222 spots in first comparison and 173 spots in second comparison. T-test was used for the detection of protein spot with significant expression between normal and stress. 49 proteins spots in Bam and 21 proteins spots in Arta were appeared. Among the 49 significantly proteins spots in Bam, 14 and 35 spots showed up-regulated and down-regulated

respectively. Among 21 significantly proteins spots in Arta, 6 and 15 spots showed up-regulated and down-regulated respectively (Fig. 1).

The proteins identified included many previously characterized stress-responsive proteins and others related to processes including metabolism of energy; scavenging for reactive oxygen species and detoxifying; protein translation, processing and degradation; Cell wall-related proteins; Amino acid metabolism and Hormone-related proteins; Signal transduction network; Cytoskeleton; Transcription-related proteins; Correlation analysis of mRNA and proteins levels (Fig. 2).

Discussion

Metabolism of energy

Under salt stress in plants to conserve energy and to limit the amount of energy metabolism decreases ROS (Moller, 2001). Previously it was reported that the frequency Transcript members involved in glycolysis, citrate cycle, respiration, mitochondrial pentose phosphate pathway is generally reduced under salt stress in Arabidopsis roots (Jiang and Deyholos, 2006).

Table 2. Nacl responsive proteins identified in Arta cultivar. [a] Spot numbers given by PDQuest software/ [b] The component increased (↑), decreased (↓)

Spot no[a]	Identified protein	pI/MW experimental	pI/MW theoretical	Express. Level[b]	Accession no
2405	Calreticulin 2 (CRT2)	4.4/50	4.37/48.4	↑	At1g09210
2406	Calreticulin 2 (CRT2)	4.3/49	4.37/48.4	↑	At1g09210
3402	Aminomethyl transferase	5.3/45	5.93/44.38	↓	SGN-U329087
3705	Lipoxygenase 1	5.4/96	5.73/96.39	↓	TC146955
4204	Lactolyglutathione lyase	5.6/31	5.51/32.55	↓	TC130772
4308	Peroxidase	5.9/37	5.91/36.55	↓	TC140370
4506	Vacuolar ATPase subunit B	5.6/52	4.96/54.13	↑	SGN-U312672
4606	Aldehyde dehydrogenase (ALDH1a)	5.8/59	6.29/54.89	↑	SGN-U319484
4706	Transketolase 1	5.6/84	6.16/80.11	↑	SGN-U312322
4716	F23N19.10 stress-include protein	5.7/76	6.24/67.32	↓	TC139384
5301	Putative nuclear RNA-binding protein A	6.2/37	6.37/40.42	↓	35_16328
5510	Cytosolic 6-phosphogluconate dehydrogenase	6.1/56	6.58/51.58	↑	TC133105
6303	Malate dehydrogenase cytosolic	6.5/40	6.10/35.74	↓	SGN-U312385
6304	Pyruvate dehydrogenase	6.6/41	6.87/43.37	↓	SGN-U315305
6402	Putative monodehydroascorbate reductase	6.3/48	6.84/52.75	↓	TC132873
6406	DNA-binding protein GBP16	6.4/50	6.48/43.15	↓	SGN-U315500
6504	Neutral leucine aminopeptidase preportein	6.3/57	7.92/60.28	↓	SGN-U312375
6510	Catalase 1	6.6/56	6.68/56.58	↓	TC139229
7302	Glyceraldehyde-3-phosphate dehydrogenase C subunit (GAPC)	6.8/43	6.62/37	↓	At3g04120
7402	Fumarase (FUM1)/fumarate hydratase	6.7/44	8.01/53.5	↓	At2g47510
7403	Pyruvate dehydrogenase	6.7/44	6.87/43.37	↓	SGN-U315305

In another study on the roots of Arabidopsis under salt stress was concluded that 11 proteins involved in glycolysis, citrate cycle, pentose pathway and electron transport is reduced, for example, aconitase, isocitrat dehydrogenase, fumarase, malate dehydrogenase four enzymes involved in the citrate cycle under salt stress reduced. In contrast, the conversion of GTP to ATP and mitochondrial malate dehydrogenase -Enzyme NDPK1 frequency in response to increased salinity. Reduce the number of LOS2 proteins (spot3503) suggests that the metabolic disorder caused by sodium chloride in glycolysis may be different from its role in cold stress (Jiang *et al.*, 2007).

Most proteins whose function is related to carbon metabolism, have shown increased expression under salt stress. The amount of the plant genotype and the back stress tolerance.

Some of the genes involved in carbohydrate metabolism in the short-term effects of sodium chloride stress or osmotic stress showed decreased expression (Jiang and Deyholos, 2006). Methylmalonate semi-aldehyde dehydrogenase (spot4101), involved in energy production, and UDP-glucose pyrophosphorylase which is related to sucrose and starch metabolism were, in control conditions, also less abundant in tolerant genotypes than in sensitive ones but were more up-regulated by salt stress. Our results showed that some proteins associated with energy production or with transport, malate dehydrogenase cytosolic (spot6303) and methylmalonate semi-aldehyde dehydrogenase (spot4101), also exhibited genotype- and salt related abundance variations. vacuolar ATPase and phosphoglycerate kinase) were less abundant after salt stress. V-ATPase (spot4506), which may provide the driving force for Na+ transport, via Na+−H+ exchangers, to isolate toxic ions within the vacuole, has been associated, in many studies, to the ability of the plant to resist salty conditions (Manaa *et al.*, 2011).

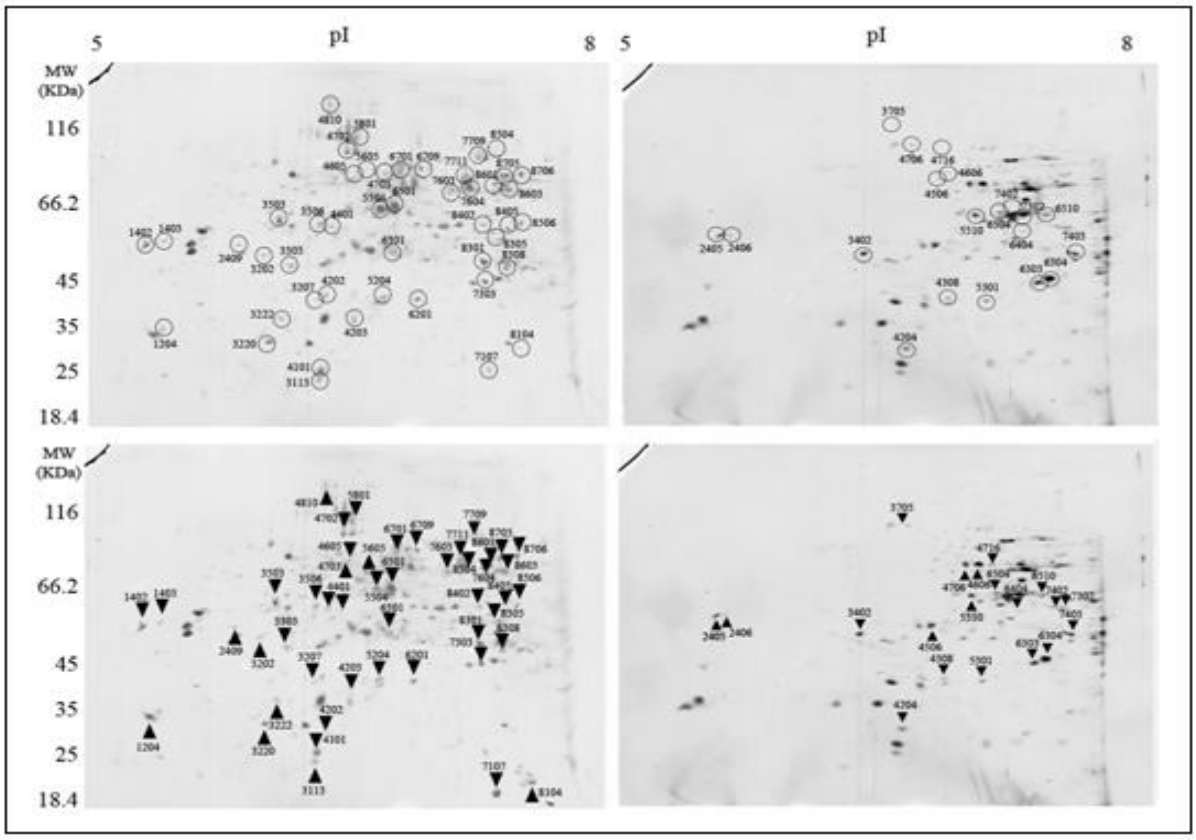

Fig. 1. The position of differentially expressed spots on 2-DE gels I tolerant cultivar, Bam (Left) and susceptible cultivar, Arta (Right) under normal (Up) and stress (Down) condition.

Scavenging for reactive oxygen species and Detoxifying

Abiotic stresses induced production of ROS that cause damage to cellular components and on the other hand, can act as signaling molecules act in response to stress (Apel and Hirt, 2004). Exposure to increased ROS production plants exposed to salt stress, which causes oxidative damage to cellular components such as membrane lipids, proteins and nucleic acids is (Apel and Hirt, 2004; Tanaka *et al.*, 2006). Plants can regulate the ROS level through complex mechanisms such as Scavenging them with ascorbate peroxidase (APX) (spot5204, 4308), glutathione peroxidase (GPX) (spot3113), glutathione S-transferase (GST), and superoxide dismutase (SOD), of which nine proteins were identified in this study (Jiang *et al.*,

2007). Other enzymes such thioredoxin important role in the immune system against oxidative damage by reducing the disulfide bonds of proteins plays oxidase. These proteins play a crucial role in the adaptation of plants to salinity conditions (Apel and Hirt, 2004; Bhushan *et al*., 2007). Suggested that some family members of APX, GPX, GST and SOD, are part of the antioxidant system used by plants (Apel and Hirt, 2004). Previous microarray results show that the members of this system answer to different stresses such as salinity, osmotic stress, cold and dry (Seki *et al*., 2002; Jiang and Deyholos, 2006). Over expression of some of APX, SOD and GST genes to improve the oxidative stress tolerance in transgenic plants is shown (Mittler, 2002). Previous microarray analysis has shown that the strongest response AtGPX6 Transcript isoforms are under abiotic stresses often play an important role in protecting against oxidative damage (Milla *et al*., 2003). SOD enzyme catalyzes the conversion of oxygen and H2O2, and formed the first line of defense against ROS are within a cell (Alscher *et al*., 2002). Surprisingly, in the previous microarray analysis, copies of all three of salinity decreased SOD gene (Jiang and Deyholos, 2006).

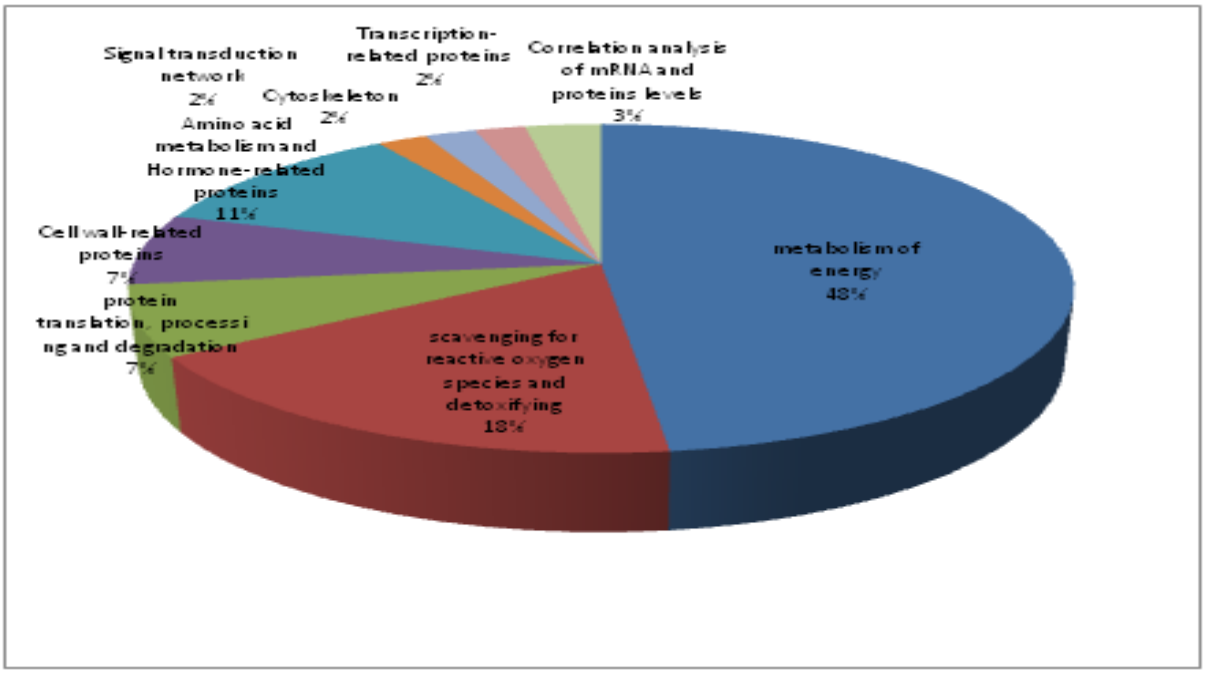

Fig. 2. Identified protein's classifying to biological functional groups.

Protein translation, processing and degradation

Regulation of gene expression in eukaryotes, especially in areas such as transcription, post-transcription, translation and post-translation takes place. Major reduction in the synthesis of new proteins in Arabidopsis was observed after treatment with sodium chloride (Ndimba *et al*., 2005). Reduce the number of MtHSC70-2 after NaCl shows that the decline in newly synthesized peptides are transported into the mitochondria, partly due to new protein synthesis under conditions of salinity (Jiang *et al*., 2007). Regulation of HSP90 in tomato treated with sodium chloride to its role in preventing the accumulation of denatured proteins and facilitate the folding back again (Chang *et al*., 2000; Wang *et al*., 2008; Chen *et al*., 2009). HSP90 gene expression in tobacco tolerate salt stress was effective (Liu *et al*., 2006).

MG toxic effects are mutant nucleic acid and protein subunits, including alteration and destruction (Thornalley, 1996). MG accumulation show plant is under stress such as salinity, drought and cold (Yadav *et al*., 2005).

Cell wall-related proteins

Salt stress reduces the amount of water available to plants and lead to the inhibition of plant growth is the increasing pressure on the eve of the wall. This pressure causes cell spread or induces hydraulic limitations to absorb water (Steudle, 2000). GH1 and GH17 family proteins (spot7604, 8506, 8601, 8603), including β- glycosidase and β-1,3-glucanase important role in many physiological processes in plants, including reconstruction of the cell wall (Bray, 2004; Xu *et al*., 2004). GRPS post-translational modifications regulate gene expression in plants under stress does play a role. In most cases, the vascular tissue accumulation and synthesis of plant defense mechanism (Mousavi and Hotta, 2005). Salinity caused a temporary increase in the frequency of AtGRP7 (Jiang *et al*., 2007). Three proteins associated with the cell wall GRP7, Caffeoyl-CoA O-methyltransferase 6 and S1UPTG1. Have important role in post-transcriptional changes in gene expression in plants under stress plays. Important role in the processes of defense against stress and protein GRP7 also reduced in the susceptible cultivar showed sustained expression (Mousavi and Hotta, 2005). Up regulation of S1UPTG1 protein in cell wall biosynthesis root occurs in response to salt stress (Li *et al*., 2008).

Amino acid metabolism and Hormone-related proteins

Jasmonic acid and ethylene, are hormones that are associated with environmental stresses (Chen *et al*., 2005; Devoto and Turner, 2005). Jasmonic acid in a wide variety of stresses, defense and developmental processes involved (Devoto and Turner, 2005). One of the enzymes involved in the biosynthesis of Jasmonic acid, AOC2 (spot1402, 1403) the frequency is increased under salt stress. It seems that the increase Jasmonic acid biosynthesis may also be associated with response to sodium chloride in Arabidopsis roots (Jiang *et al*., 2007). Some amino acids like proline content increased after treatment with sodium chloride (Di Martino *et al*., 2003). Ashraf and Harris (2004) found that stress causes the accumulation of free amino acids such as glutamine hyper osmotic, Sparzhyn and proline in plant cells. The frequency of amino acid 3-Isopropylmalate dehydrogenase and Cobalamine-independent methionine syntethase decreased after treatment with sodium chloride. But Frequency of glutamate dehydrogenase 2 (GDH2) (spot8301) and glutamine synthetase (GS) in the first six hours of salt stress reduction and increased after 48 hours (Jiang *et al*., 2007). GS and GDH with a number of other enzymes, plays a key role in maintaining the balance of carbon and nitrogen (Miflin and Habash, 2002). Studies show that salinity signal to produce ROS, causing α-GDH subunit expression and anionic iso-GDH as anti-stress enzymes in the detoxification of ammonia and glutamine are reproducible (Skopelitis *et al*., 2006). SAMS, isopropyl malate dehydrogenase and glutamine synthetase has effect on amino acid synthesis and osmotic adjustment in roots under salt stress (Ouyang *et al*., 2007).

Signal transduction network

Increase the concentration of sodium chloride in the extracellular space can be assumed by the sensor in the cell membrane to regulate gene expression in Arabidopsis and transfer the cellular understood (Chinnusamy *et al*., 2005). Stress can disable stimulation of the formation ROS in the Ca^{2+} transmission path, central regulators and can affect on plant growth factors (Mortimer *et al*., 2008). Proteins such CRT1 and CRT2 (spot2406), protein associated with calcium and small Ras-like GTP-binding (Ran-1) protein in signal transduction networks are involved in salt stress (Jiang *et al*., 2007). CRT1 and CRT2 major isoforms that link to Ca^{2} plays an important role in Ca^{2+} homeostasis under environment.al stress (Yang, 2002). There is little information about the function of (Ran) in plant response to stress. Ran1 frequency after 48 hours of treatment with sodium chloride is increased (Jiang *et al*., 2007).

Cytoskeleton

Actin (spot6301) and tubuline have important

applications in cell homeostasis. Cytoskeleton reconstructed by endogenesis and external stimuli such as hormones, low temperature, aluminum, sodium chloride (Sivaguru *et al.*, 2003). Research shows that salt stress impairs cortical microtubules array through the SPR1.Role of (SPR1) is inhibition of dissimilar growth (Shoji *et al.*, 2006). It was found that the prevalence of the actin protein (ACT8) and a β chain tubuline decreased under salt stress. While the tubuline, chain 6-α (TUA6) is induced by sodium chloride. However, their importance is not entirely clear (Jiang *et al.*, 2007).

Transcription-related proteins

The protein consist of NAC domain (spot8104) in Arabidopsis root is similar to humans transcription factors (BTF3) under NaCl stress (Jiang *et al.*, 2007). Down-regulation of BTF3 in *Nicotiana benthamiana* cause reduction of chloroplast and mitochondrial and chloroplast genes were expressed and that in these cells were producing large amounts of ROS (Yang *et al.*, 2007).

Correlation analysis of mRNA and proteins levels

The relationship between gene expression at the mRNA (spot1402, 1403) level and protein is not clear in the root tissues of plants under abiotic stresses (Jiang and Deyholos, 2006). The relationship between expression ratios (For example, treatment /control) Observed in previous experiments for protein and transcript has been rarely reported and this measure represents the mRNA is not associated with protein frequency (Yan *et al.*, 2006).

Conclusion

Two wheat cultivars with different tolerance to response NaCl stress was compared in term of molecular responses using two dimensional polyacrilamid gel electrophoresis that allow monitoring of proteome changes and could identify some proteins that have key role in wheat response to Nacl stress. These proteins are involved in main metabolism of energy and scavenging for reactive oxygen species and detoxifying. Investigating them could help to understand molecular basis of wheat response to NaCl stress.

Reference

Abdul Kader MD, Lindberg S. 2010. Cytosolic calcium and pH signaling in plants under salinity stress. Plant Signaling and Behavior **5,**233-238.

Alscher RG, Erturk N, Heath LS. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Journal of Experimental Botany **53,** 1331–1341.

Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology **55,** 373–399.

Bhushan D, Pandey A, Choudhary MK, Datta A, Chakraborty S, Chakraborty N. 2007. Comparative proteomics analysis of differentially expressed proteins in chickpea extracellular matrix during dehydration stress. Molecular and Cellular Proteomics **6,** 1868–1884.

Bray EA. 2004. Genes commonly regulated by water-deficit stress in Arabidopsis thaliana. Journal of Experimental Botany **55,** 2331–2341.

Chang WWP, Huang L, Shen M, Webster C, Burlingame AL, Roberts JKM. 2000. Patterns of protein synthesis and tolerance of anoxia in root tips of maize seedlings acclimated to a low-oxygen environment, and identification of proteins by mass spectrometry. Plant Physiology **122,** 295–318.

Chen S, Gollop N, Heuer B. 2009. Proteomic analysis of saltstressed tomato (*Solanum lycopersicum*) seedlings: effect of genotype and exogenous application of glycinebetaine. Journal of Experimental Botany **60,** 2005–2019.

Chen YF, Etheridge N, Schaller GE. 2005. Ethylene signal transduction. Annals of Botany 95, 901–915.

Chinnusamy V, Jagendorf A, Zhu JK. 2005. Understanding and improving salt tolerance in plants. Crop Science **45,** 437-448.

Devoto A, Turner JG. 2005. Jasmonate-regulated Arabidopsis stress signalling network. Physiologia Plantarum **123,** 161–172.

Di Martino C, Delfine S, Pizzuto R, Loreto F, Fuggi A. 2003. Free amino acids and glycine betaine in leaf osmoregulation of spinach responding to increasing salt stress. New Phytologist **158,** 455–463.

Gao L, Yan XLi, X, Guo G, Hu Y, Ma W, Yan Y. 2011. Proteome analysis of wheat leaf under salt stress by two-dimensional difference gel electrophoresis (2D-DIGE). Phytochemistry **72,** 1180-1191.

Jiang Y, Deyholos M. 2006. Comprehensive transcriptional profiling of NaCl-stressed Arabidopsis roots reveals novel classes of responsive genes. BMC Plant Biology **6,** 25.

Jiang Y, Yang B, Harris NS, Deyholos MK. 2007. Comparative proteomic analysis of NaCl stress-responsive proteins in Arabidopsis roots. Journal of Experimental Botany **58,** 3591-3607.

Jiang YQ, Deyholos MK. 2006. Comprehensive transcriptional profiling of NaCl-stressed Arabidopsis roots reveals novel classes of responsive genes. BMC Plant Biology **6,** 25.

Joseph B, Jini D. 2010. Proteomic analysis of salinity stress-responsive proteins in plants. Asian Journal of Plant Sciences **9,** 307-313.

Li J, Wu XD, Hao ST, Wang XJ, Ling HQ. 2008. Proteomic response to iron deficiency in tomato root. Proteomics **8,** 2299–2311.

Liu DL, Zhang XX, Cheng YX, Takano T, Liu SK. 2006. rHsp90 gene expression in response to several environmental stresses in rice (*Oryza sativa* L.). Plant Physiology and Biochemistry **44,** 380–386.

Manaa A, Ahmed HB, Valot B, Bouchet JP, Aschi-smiti S, Causse M, Faurobert M. 2011. Salt and genotype impact on plant physiology and root proteome variations in tomato. Journal of Experimental Botany 1-17 p.

Miflin BJ, Habash DZ. 2002. The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. Journal of Experimental Botany **53,** 979–987.

Milla MA, Maurer A, Huete AR, Gustafson JP. 2003. Glutathione peroxidase genes in Arabidopsis are ubiquitous and regulated by abiotic stresses through diverse signaling pathways. The Plant Journal **36,** 602–615.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science **7,** 405–410.

Moller IM. 2001. Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annual Review of Plant Physiology and Plant Molecular Biology **52,** 561–591.

Mortimer JC, Laohavisit A, Macpherson N, Webb A, Brownlee C, Battey NH, Davies JM. 2008. Annexins: multifunctional components of growth and adaptation. Journal of Experimental Botany **59,** 533–544.

Mousavi A, Hotta Y. 2005. Glycine-rich proteins: a class of novel proteins. Applied Biochemistry and Biotechnology **120,** 169–174.

Ndimba BK, Chivasa S, Simon WJ, Slabas AR. 2005. Identification of Arabidopsis salt and osmotic stress responsive proteins using two dimensional difference gel electrophoresis and mass spectrometry. Proteomics **5,** 4185–4196.

Ouyang B, Yang T, Li H, Zhang L, Zhang Y, Zhang J, Fei Z, Ye Z. 2007. Identification of early salt stress response genes in tomato root by suppression subtractive hybridization and microarray analysis. Journal of Experimental Botany **58,** 507–520.

Saqib M, Zorb C, Schubert S. 2006. Salt-resistant and salt-sensitive wheat genotypes show similar biochemical reaction at protein level in the first phase of salt stress. Journal of Plant Nutrition and Soil Science **169,** 542-548.

Seki M, Narusaka M, Ishida J. 2002. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. The Plant Journal **31,** 279–292.

Shoji T, Suzuki K, Abe T, Kaneko Y, Shi H, Zhu JK, Rus A, Hasegawa PM, Hashimoto T. 2006. Salt stress affects cortical microtubule organization and helical growth in Arabidopsis. Plant and Cell Physiology **47,** 1158–1168.

Sivaguru M, Pike S, Gassmann W, Baskin TI. 2003. Aluminum rapidly depolymerizes cortical microtubules and depolarizes the plasma membrane: evidence that these responses are mediated by a glutamate receptor. Plant and Cell Physiology **44,** 667–675.

Skopelitis DS, Paranychianakis NV, Paschalidis KA, Pliakonis ED, Delis ID, Yakoumakis DI, Kouvarakis A, Papadakis AK, Stephanou EG, Roubelakis-Angelakis KA. 2006. Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. The Plant Cell **18,** 2767–2781.

Song X. 2007. Wheat (*Triticum aestivum* L.) root proteome and differentially expressed root proteins between hybrid and parents. Proteomics **7,** 3538-3557.

Steudle E. 2000. Water uptake by roots: effects of water deficit. Journal of Experimental Botany **51,** 1531–1542.

Tanaka A, Christensen MJ, Takemoto D, Park P, Scott B. 2006. Reactive oxygen species play a role in regulating a fungus–perennial ryegrass mutualistic interaction. The Plant Cell **18,** 1052–1066.

Thornalley PJ. 1996. Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification—a role in pathogenesis and antiproliferative chemotherapy. General Pharmacology **27,** 565–573.

Tuteja N. 2007. Mechanisms of high salinity tolerance in plants. Methods in Enzymology **428,** 419-438.

Wang MC, Peng ZY, Li CL, Li F, Liu C, Xia GM. 2008. Proteomic analysis on a high salt tolerance introgression strain of *Triticum aestivum/Thinopyrum ponticum*. Proteomics **8,** 1470–1489.

Xu Z, Escamilla-Trevino L, Zeng L. 2004. Functional genomic analysis of *Arabidopsis thaliana* glycoside hydrolase family 1. Plant Molecular Biology **55,** 343–367.

Yadav SK, Singla-Pareek SL, Ray M, Reddy MK, Sopory SK. 2005. Methylglyoxal levels in plants under salinity stress are dependent on glyoxalase I and glutathione. Biochemical and Biophysical Research Communications **337,** 61–67.

Yan SP, Zhang QY, Tang ZC, Su WA, Sun WN. 2006. Comparative proteomic analysis provides new insights into chilling stress responses in rice. Molecular and Cellular Proteomics **5,** 484–496.

Yang KS, Kim HS, Jin UH, Lee S, Park JA, Lim

Y, Pai HS. 2007. Silencing of NbBTF3 results in developmental defects and disturbed gene expression in chloroplasts and mitochondria of higher plants. Planta **225,** 1459–1469.

Yang Z. 2002. Small GTPases: versatile signaling switches in plants. The Plant Cell (Suppl.) **14,** S375–S388.

Zhao GQ, Ma BL, Ren CS. 2007. Growth, gas exchange, chlorophyll fluorescence, and ion content of naked oat in response to salinity. Crop Science **47,** 123-131.