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Proteomics analysis of canola seeds to identify differentially expressed proteins under salt stress

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Abstract
Seeds are an important part of the canola plant, and similar to other parts are affected by salt stress. Understanding the underlying mechanisms that take place in seeds of canola under salt stress is essential from the perspective of improving quality. In this study, we attempted to identify differentially expressed proteins of canola seeds in the Hyola308 cultivar under 350 mM NaCl using two dimensional (2D) gel electrophoresis. Effect of salt stress was significant on 100-seed weight and number of seeds per plant ($p \leq 0.01$), and it changed the proteome of the seeds. From a total of 548 reproducible protein spots, 28 protein spots showed significant changes in abundance, of which seven spots showed downregulation. The "Gene Ontology" analysis classified differentially expressed proteins into six biological processes: oxidation-reduction (28.5%), response to abiotic stress (28.5%), response to hormones (21.4%), catabolic process (21.4%), nucleoside diphosphate phosphorylation (17.8%) and glycolytic process (14.28%). In conclusion, salt stress induced canola seeds to upregulate proteins that mostly involved in the antioxidant activity and the proteins with nutrient reservoir activity.

Keywords: Abiotic stress; Antioxidant activity; *Brassica napus*; Proteomics; Two-dimensional polyacrylamide gel electrophoresis.

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Introduction

Global population grows constitutively leading to demand for 70% more food, feed and fuel by 2050 worldwide (FAO 2009). Salinity is one of the important abiotic stresses that challenges agricultural production. It affects more than 20% of the cultivated land worldwide. The affected regions are increasing day by day (Gupta and Huang 2014). Salt stress as an important environmental stress affects plant growth similar to other types of stresses, but what differentiate salt stress from other stresses, is its effect on plants during the entire course of growth and development. In the other word, other stresses only affect plants in some part of their growth stages. The plants grown under salt conditions are unable to absorb adequate water because of osmotic pressure so they experience water deficiency (Kaya *et al.* 2006) in addition to accumulation of toxic ions (Munns and Tester 2008). Under saline conditions, the plant cell division and expansion are impaired (Hasegawa *et al.* 2000), resulting in physiological and biochemical alteration (Parida and Das 2005) and decreasing the plant growth (Munns and Tester 2008).

Canola (*Brassica napus* L.) is one of the widely cultivated oil crops for producing vegetable oil. Canola have the healthy fatty acid composition (Ghazani and Marangoni 2016) and high protein content (Rutkowski 1971) which make this plant an attractive crop for large-scale production of edible-vegetable oil and biofuel (Ghazani and Marangoni

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2016). The rapeseed plant tolerates abiotic stresses; however, its growth and yield are adversely affected (Purty *et al.* 2008).

Salt stress are threating *B. napus* similar to other crops especially in arid and semi-arid regions. Seeds are the most important parts of the canola plant. Although seeds are not directly in contact with salt stress, understanding changes in protein networks in response to salt stress is remarkably vital from the perspective of improving oil yield and quality.

Proteomics approaches allow researchers to study mixtures of proteins and collect a large amount of information about proteins involved in a specific biological response. This approach has widely been used for analyzing seed proteome profile changes during germination and aging in different plants (Chibani *et al.* 2006; Gallardo *et al.* 2007; Brandão *et al.* 2010). Studies using different approaches of proteomics have indicated that functional seed proteins categorized in several categories such as central metabolism, cellular structure, stress response, nucleic acid metabolism, protein synthesis, protein folding, protein targeting, hormones and signaling, membrane transport and proteins of unknown function (Finnie *et al.* 2002; Vensel *et al.* 2005; Miernyk and Hajduch 2011; Miernyk *et al.* 2011). Brandão *et al.* (2010) reported that about 18 of 350 seed protein spots of soybean exposed to salt stress showed changes in expression. They demonstrated that ferritin and 20S proteasome subunit β-6 were upregulated, whereas glyceraldehyde 3-phosphate dehydrogenase, glutathione S-transferase (GST) 9, GST 10 and seed maturation protein PM36 were downregulated. Proteins involved in the glycolysis and carbohydrate metabolism, as well as stress-related proteins are differentially expressed in seeds of durum wheat in response to heat stress (Laino *et al.* 2010).

Several studies have been conducted about proteomic analysis of canola leaf and root (Bandehagh *et al.* 2011; Banaei-Asl *et al.* 2015; Shokri Gharelo *et al.* 2016); however, there are limited reports about proteome analysis of canola seeds in response to salinity. Therefore, in this investigation we attempted to determine differentially expressed proteins of canola seeds, and possible mechanisms by which seeds respond to salt stress.

Material and Methods

Plant materials and growth conditions

Canola seeds of the Hyola308 cultivar were provided from Seed and Plant Improvement Institute (SPII), Karaj, Iran. Seeds were germinated and planted in greenhouse conditions at the research greenhouse of University of Tabriz, Tabriz, Iran. Seeds were sterilized by 70% ethanol and 1% hypochloride sodium followed by washing them by distilled water. After germination in distilled petri dishes, the seeds were transplanted and grown in the hydroponic system under the following conditions: 50% and 60% humidity during day and night, respectively, temperature of 25±2 °C, 14 h of light and feeding with sterile Hoagland's solution optimized for canola (Bandehagh *et al.* 2011).

Experimental design and salt stress treatment

A completely randomized design with three replications was carried out, in which salt stress at

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three levels (0, 175 and 350 mM NaCl) was imposed on plants. The seeds from the control plants and those treated with 350 mM NaCl, were harvested at maturity and used for proteomics analysis. Also, the number of seeds per plant and 100-seed weight were measured for the plants treated with the three levels of salt stress.

Two-dimensional (2D) gel electrophoresis

The method described by Finnie *et al.* (2002) followed to extract proteins from the seeds. Seeds were ground and 4 gr of grounded seeds were added to 20 ml extraction buffer (5 mM Tris, pH 7.5; 1 mm $CaCl_2$). Powdered seeds were mixed with extraction buffer for 30 minutes. Centrifugation with 16000 RCF, at 4 ºC for 30 minutes, was performed to collect the pellet. All protein extraction stages were done under 4 ºC temperature. Protein concentration was determined according to Zor and Selinger (1996) using the Bradford (1976) method.

To separate the extracted proteins in the first dimension, immobilized pH gradient (IPG) strips with 18 cm length (Bio-Rad) and 3-10 pH gradient were rehydrated using rehydration buffer (2 M thiourea, 7 M urea, 2% dithiothreitol (DTT), 4% CHAPS and 2% pharmalyte). Isoelectric focusing (IEF) was carried out as follows: 300 V for 1.5 h, 500 V for 1.5 h, 1000 V for 1.5 h, 3500 V for 2 h, and 10000 V for 1.5 h. The focused strips were equilibrated twice for 15 minute in the solution containing 72.07 gr of urea, 69 ml of glycerol, 0.1 gr of DTT and 4 gr of SDS. In the second equilibration, iodoacetamide was used instead of 0.1g DTT to prevent the reformation of disulfide bonds. Equilibrated IPG strips were transferred to the top of stacking gel with 40% polyacrylamide gel to operate the second dimension, using PROTEIN II 11 Multicell (Bio-Rad). Focusing was carried out with 250 V for 30 minute, 10000 V for 2.5 h and 10000 V for 40000 V-h conditions. The gels were stained by the silver staining method (Sammons *et al.* 1981). The GS-800 densitometer (Bio-Rad) was used for the gel scanning.

In order to find differentially expressed protein spots of canola under salt stress, gels from control plants and plants grown under 350 mM NaCl were comparatively examined using Melanie 4 (GeneBio, Geneva, Switzer land). The relative intensity of protein spots was used for finding protein spots with expression changes. Those protein spots that had induction factor of more than 2-fold intensity regarded as upregulated proteins and those had less than 0.5-fold regarded as downregulated proteins.

Reproducible protein spots with the significant change in expression were selected and identified, using their isoelectric point and molecular weight. Spots' molecular weight was determined with the protein marker that was loaded with the solved proteins samples in the second dimension. Protein database sites, including NCBI, Expassy, Wheat proteomics, TAIR and UniProtKB were surfed for the identification of protein spots.

Gene Ontology (GO) analysis of differentially expressed proteins.

The BiNGO (The Biological Networks Gene Ontology tool) App in the Cytoscape data base (https://cytoscape.org/), was used to study and visualize biological pathways and molecular functions of the proteins (Shannon *et al.* 2003).

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This tool calculates overrepresented GO terms in the network and display them as a network of significant GO terms (Maere *et al.* 2005). BiNGO was set with the following parameters: hypergeometric test selected for the statistical test, 0.01 probability level selected for a significance threshold and *Arabidopsis thaliana* selected for organism/annotation. To annotate the biological process of protein spots, homologous proteins of these differentially expressed proteins were found in the well-studied *Arabidopsis* plant at TAIR database (https://www.arabidopsis.org/). TAIR allows to search for protein information using a variety of parameters.

Statistical analysis

To analyze the data for 100-seed weight and number of seeds per plant, one-way analysis of variance was carried out. Then, the treatment means for these traits were compared by Duncan's multiple range test. To compare the means of control with 350 mM NaCl, obtained from the proteomics analysis, Student's t-test was used.

Results

Effects of salt stress on 100-seed weight and number of seeds per plant

Effect of salt stress on 100-seed weight and number of seeds per plant of canola was significant Figure1). The results showed that 100-seed weight of canola decreased under 350 mM NaCl compared to the control plants, while there was no significant difference between salinity treatments (175 and 350 mM NaCl). Both salinity treatments decreased number of seeds per plant significantly compared with the control. However, the decrease under 350 NaCl was significantly larger than 175 mM NaCl.

Quantitative analysis of differentially expressed proteins

Silver staining visualized 548 reproducible protein spots, of which a total of 28 spots indicated differentially expression changes in response to salt stress (Figure 2). Of 28 spots with significant changes in expression, seven protein spots showed downregulation and 21 spots showed upregulation (Table 1).

GO analysis of differentially expressed proteins

As represented in Figure 3, these proteins belonged to the following biological process: oxidation-reduction process (28.5%), response to abiotic stress (28.5%), response to hormone (21.4%), catabolic process (21.4%), nucleoside diphosphate phosphorylation (17.8%) and glycolytic process (14.28%).

Analysis of data indicated that eight protein spots with altered expression level were related to oxidation-reduction process; catalase isozome 1 (spot No. 8), glucose and ribitol dehydrogenase (spot No. 5), annexin 2 (spot No. 27), rubulose-1, 5-bisphosphte (spot No. 22), glyceraldehyde 3-phosphate (spot No. 2) and peroxidase 1 (spot No. 6). Except for spot No. 22 that showed downregulation, other protein spots of this category showed upregulation in response to salt stress.

Proteins involved in response to abiotic stress were glutathione peroxidase 1 (spot No. 6), serpin-Z2B (spot No. 7), catalase isozome 1 (spot No. 8),

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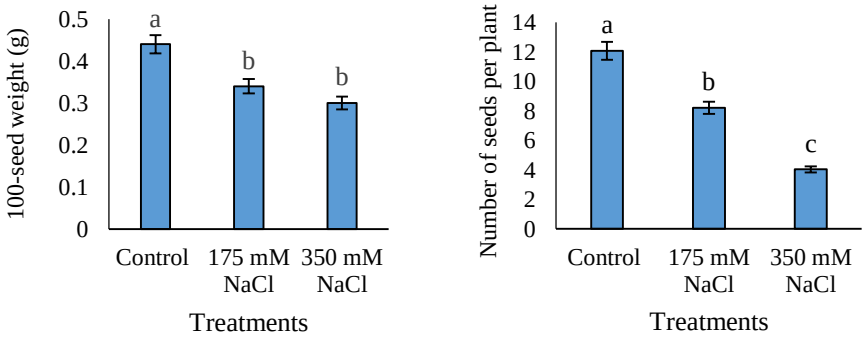

Figure 1. The effect of salinity level on 100-seed weight and number of seeds per plant in canola, cv. Hyola308; Treatments with different letters represent statistically significant difference, according to Duncan's multiple range test.

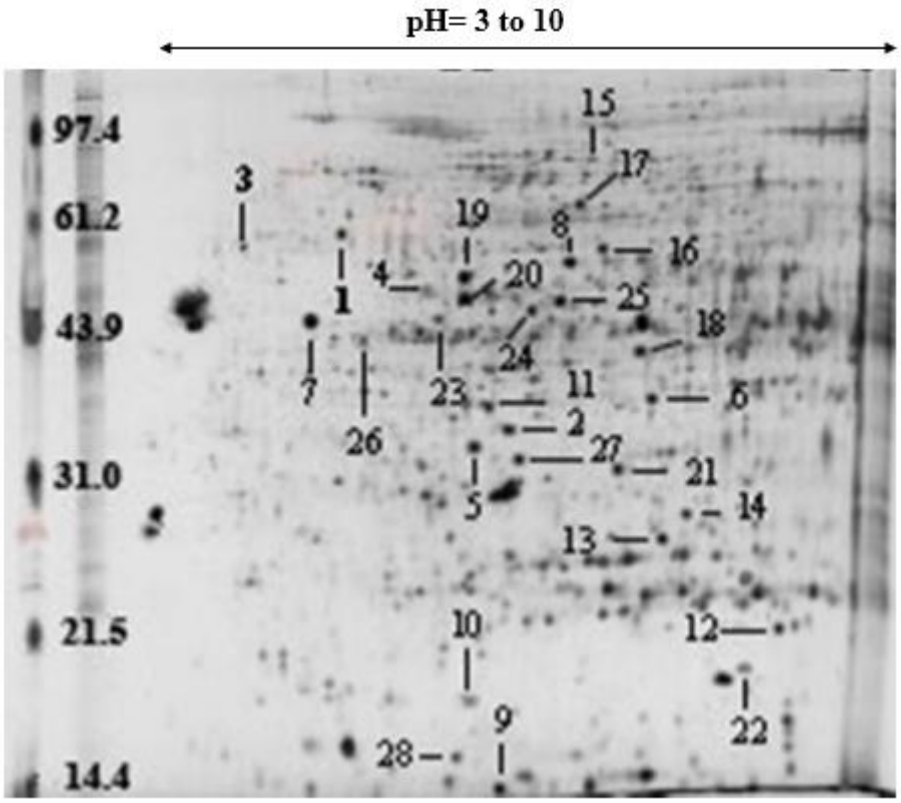

Figure 2. 2D electrophoresis gel of canola, cv. Hyola308, seed extract. 120 µg of proteins were extracted and separated in 3 to 10 ranges of pH, followed with resolving proteins in 40% polyacrylamide gel. Gels were stained by silver. Identified proteins were numbered on the reference gel.

alpha-amylase/trypsin (spot No. 9), Os05g0453700 (spot No. 10), Os07g0683900 (spot No. 11), group 3 late embryogenesis (spot No. 12) and basic endochitinase C (spot No. 12). Of these spots, No. 10 showed downregulation, while remaining proteins showed upregulation.

In the category of response to hormone, β amylase (spot No. 1), glyceraldehyde 3-phosphate (spot No. 2), and glucose and ribitol dehydrogenase (spot No. 5) were expressed at a higher level. The protein spots xylose isomerase (spot No. 3), NADPH-dependent oxidoreductase (spot No. 4),

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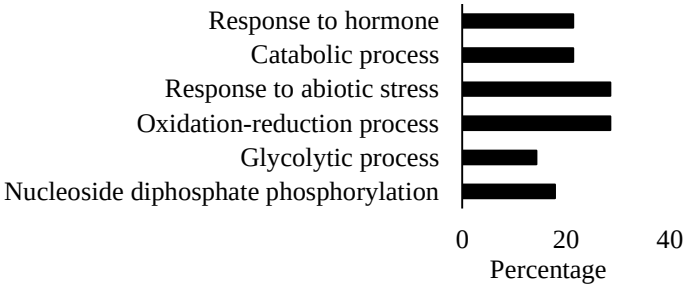

Figure 3. Distribution of seed proteins of canola, cv. Hyola308. The 28 identified protein spots from canola seeds were classified according to their biological process using GO analysis. One protein can be present in more than one category.

Table 1. List of identified differentially expressed protein spots of canola, cv. Hyola308, in the presence of 350 mM NaCl. Only spots with more than 2-fold intensity and less than 0.5-fold intensity compared to control gels considered as statistically significant protein spots with changes in expression.

No.	Description	Protein ID*	pI-MW (KDa)	IF**	Changes in expression
1	β amylase	A0A1P8B7H0	5.41-51.14	2.87	Upregulation
2	Glyceraldehyde3-phosphate	P25858	6.62-36.91	6.54	Upregulation
3	xylose isomerase	Q9FKK7	5.59-53.71	5.56	Downregulation
4	NADPH-dependent oxidoreductase	Q39172	5.80-38.13	7.14	Downregulation
5	Glucose and ribitol dehydrogenase	Q9MA93	6.09-31.45	6.95	Upregulation
6	Glutathione peroxidase1	P0DI10	9.35-35.62	8.12	Upregulation
7	Serpin-Z2B	P93692	5.18-42.98	5.98	Upregulation
8	Catalase isozome1	Q96528	6.95-56.76	7.32	Upregulation
9	Alpha-amylase/trypsin	Q8VZ56	5.62-47.37	4.10	Upregulation
10	Os05g0453700	Q7XXS5	6.29-17.93	4.17	Downregulation
11	Os07g0683900	Q7XXS5	6.29-17.91	3.54	Upregulation
12	Group3late embryogenesis	Q9FG31	9.43-16.17	5.20	Upregulation
13	Basic endochitinase C	P19171	7.81-36.18	3.13	Upregulation
14	Chain A, Crystal structure of Xylanase	Q39026	7.62-29.3	3.05	Upregulation
15	Vicilin-like seed storage protein	Q9LUJ7	6.64-55.6	4.56	Upregulation
16	Vicilin-like seed storage protein	Q9LUJ7	6.64-55.6	7.32	Upregulation
17	Vicilin-like seed storage protein	Q9LUJ7	6.64-55.6	6.54	Upregulation
18	Triticin precursor	Q08837	9.37-56.93	6.87	Upregulation
19	Triticin precursor	Q08837	9.37-56.94	5.20	Upregulation
20	Triticin precursor	Q08837	9.37-56.95	4.50	Upregulation
21	Avenin- like protein	Q2A783	8.08-32.72	3.98	Upregulation
22	Rubulose-1, 5-bisphosphte	P10795	7.59-20.21	2.94	Downregulation
23	Formate dehydrogenase	A0A1P8B9N1	6.17-39.63	8.33	Downregulation
24	Aspartate aminotransferase	P37833	7.75-44.05	4.38	Upregulation
25	Aspartate aminotransferase	P37833	7.75-44.06	6.49	Upregulation
26	Glutamine synthetase isoform GSr1	F4ID91	5.14-40.39	3.33	Downregulation
27	Annexin 2	Q9SYT0	5.21-36.20	8.10	Upregulation
28	Nucleoside diphosphate kinase	O64903	9.14-25.55	2.00	Downregulation

*Identifier code at UniProtKB; http://www.uniprot.org.
**IF; Induction factor (2-fold < IF < 0.5-fold).

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and formate dehydrogenase (spot No. 23) showed downregulation.

All the proteins classified in the catalytic process were expressed at higher level (more than 2-fold) compared to control. These proteins were vicilin-like seed storage proteins (spots No. 15, 16 and 17) and triticin precursor (spots No. 18, 19 and 20).

Glutamine synthetase isoform GSr1 (spot No. 26) and nucleoside diphosphate kinase (spot No. 28) with downregulating pattern and aspartate aminotransferase (spots No. 24 and 25) with upregulating pattern were classified in the glycolytic process.

In the category of nucleoside diphosphate phosphorylation, three proteins including NADPH-dependent oxidoreductase (spot No. 4), formate dehydrogenase (spot No. 23) and nucleoside diphosphate kinase (spot No. 28) showed downregulation and glyceraldehyde 3-phosphate (spot No. 2), and glucose and ribitol dehydrogenase (spot No. 5) were upregulated in response to salt stress.

Discussion

Seeds are the most important part of canola because they are sources of oil. As a matter of fact, the main purpose of canola cultivation is the vegetable oil production (Gunstone 2011). The seed proteome of many economically crops have been described in the past. Many of those studies, however, described the proteome changes of the plants during developmental stages (Catusse *et al.* 2008; Laino *et al.* 2010; Bykova *et al.* 2011; Herman 2014). Here, we tried to identify differentially expressed proteins of canola seeds (cv. Hyola308) under salt stress to unravel the molecular mechanism taking place under these conditions.

Salinity stress at pod filling stage can cause a decrease in the photosynthetic product mobilization to grains (Chaves *et al.* 2009) and thereby decrease grain weight. A decrease in grain yield of chickpea (Singh *et al.* 1994) and grain weight, number of grains per plant, and grain, protein and oil yield of soybean (Ghassemi-Golezani *et al.* 2009) under salt stress have been reported. These results are in concordance with our results in canola seeds under 350 mM NaCl.

Proteins related to oxidation-reduction process

Production of reactive oxygen species (ROS) are induced by salt stress, leading to oxidative stress, damaging cellular components and disturbing redox hemostasis (Suzuki *et al.* 2012). Therefore, proteins involved in oxidation-reduction process of canola seeds may be induced to cope with ROS production. Our results indicated that ribulose-1, 5-bisphosphte (spot No. 22) expression was at lower level. This protein can be a regulator of ribulose-bisphosphate carboxylase (Portis 1992). Glyceraldehyde 3-phosphate dehydrogenase (spot No. 2) is a key enzyme in glycolysis that converts D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate. It was also classified as a protein involved in response to hormone and in the nucleoside diphosphate phosphorylation category. The function of this enzyme is essential for the maintenance of cellular ATP levels. Under oxidative stress, glyceraldehyde 3-phosphate dehydrogenase in association with abscisic acid triggers production of phosphatidic acid, a stress signaling molecule (Sirover 2011).

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Glucose and ribitol dehydrogenase (spot No. 5) has oxidoreductase activity and may act in signal transduction (Persson and Kallberg 2013). This protein similar to spot No. 2 was classified in the category of response to hormone and nucleoside diphosphate phosphorylation. Glutathione peroxidase1 (spot No. 6) and catalase isozyme 1 (spot No. 8) are two important antioxidant enzymes. These enzymes scavenge ROSs, preventing damages on cellular components and structures (Rhee *et al.* 2005). Annexin 2 (spot No. 27) has a peroxidase activity involving in oxidative stress. It catalyzes the reaction in which hydrogen atom from one donor is transferred on the hydrogen peroxide, producing oxidized donor and two water molecules (Konopka-Postupolska *et al.* 2011).

Proteins related to response to abiotic stress

Except Os05g0453700 (spot No. 10), the remaining proteins involved in response to abiotic stress showed upregulation. The proteins responsive to abiotic stress refer to those proteins that their abundance changes significantly under drought, salinity, high or low temperature and other sever environmental conditions (Wang *et al*. 2003). The proteins Os05g0453700 (spot No. 10) and Os07g0683900 (spot No. 11) are putative uncharacterized proteins involved in response to stress (Aki *et al*. 2008). Serpin-Z2B (spot No. 7) prevents the function of serine-type endopeptidase that catalyze hydrolysis of internal peptide bonds in a polypeptide chain (Bao *et al*. 2018). Upregulation of this protein could increase the tolerance of plants to stressful conditions (Zhou *et al*. 2016) and stop degradation of proteins in seeds (Vensel *et al*. 2005). Alpha-amylase/trypsin (spot No. 9) hydrolyzes (1-,4)-alpha-D-glucosidic linkages in polysaccharides (Panteghini and Bais 2012). Breakdown of polysaccharide takes place when seeds need energy for their activities. Group 3 late embryogenesis (spot No. 12) involves in adaptive response to water shortage (Battaglia and Covarrubias 2013). Overexpression of these types of proteins confers plant tolerance to drought stress. Water deficit is a side effect of salt stress imposed on plants in addition to the main effects of salinity. Basic endochitinase C (spot No. 13) defends plants mainly against fungal pathogens. In addition, it responds to ion toxicity that is observed commonly in the salt stress conditions (Samac *et al.* 1990).

Proteins related to response to hormone

Abscisic acid is a phytohormone that regulates various processes in the plant, such as seed dormancy, germination, senescence and response to external stresses. The hormone is believed to be an important signaling molecule in the response of plants to stress (Sreenivasulu *et al.* 2007). β-amylase (spot No. 1) breaks down (1-4)-alpha-D-glucosidic linkages in polysaccharides from the non-reducing ends of chains (Bijttebier 2008). β-amylase activity is negatively correlated with abscisic acid concentration (Wei *et al.* 2009). Xylose isomerase (spot No. 3) catalyzes D-xylose to D-xylulose involving in pentose-phosphate shunt (Katz *et al.* 2006). NADPH-dependent oxidoreductase (spot No. 4) detoxifies reactive carbonyls and formate dehydrogenase (spot No. 23) involves in the cell stress response catalyzing oxidation of formate to carbon dioxide. β-amylase (spot No. 1) expression upregulated in seeds of

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canola by salt stress, while spots 3, 4 and 23 downregulated. Molecular base of interaction of these proteins with hormones is unclear. Since abscisic acid is the predominant hormone in response to stress, we think that these proteins maybe in relationship with abscisic acid production.

Other proteins (catabolic process, nucleoside diphosphate phosphorylation and glycolytic process)

Nucleoside diphosphate phosphorylation is a process in which a phosphate group is incorporated into a nucleoside diphosphate, such as ADP, to produce a nucleoside triphosphate. Nucleoside diphosphate kinase (spot No. 28) catalyzes the transfer of terminal phosphoryl group from nucleoside triphosphate to nucleoside diphosphate (Parks and Agarwal 1973) and may involve in MAP kinase signaling (Moon *et al*. 2003). Downregulation of this enzyme could represent a decrease in nucleoside triphosphates production, such as ATP, in seeds of canola in response to salt stress.

Catabolic processes provide energy for the cell use by breaking down large molecules. Catabolic processes are mostly occurred reactions under limited nutritional conditions similar to stressful conditions. The processes could be central to plants for surviving under stress (Clifton *et al.* 2005; Millar *et al.* 2011). However, they are energy consuming reactions leading to depletion of ATPs in the long term (Tiwari *et al.* 2002). In the absence of ATPs and nutrition, the cell has to consume its own main components. This is manifested in the decreased morphological characteristics and lower performance (Farooq *et al.* 2009; Araújo *et al.* 2011). Vicilin-like seed storage protein (spots No. 15, 16 and 17) and triticin (spots No. 18, 19 and 20) are nutrient reservoir proteins. Different positions of these proteins on the gel could be due to posttranslational modifications. Results show that canola seeds increase these storage proteins in response to salt stress.

Studies on proteomics analysis of different plant tissues under stressful conditions have demonstrated that proteins related to amino acid metabolism are significantly changed (Bhushan *et al.* 2007; Banaei-Asl *et al.* 2015). Aspartate aminotransferase (spots No. 24 and 25) and glutamine synthetase isoform GSr1 (spot No. 26) are one of the main enzymes in aspartate and glutamine metabolic processes. Aspartate aminotransferase was upregulated by salt stress in contrast to the downregulated glutamine synthetase isoform GSr1. Aspartate is an intimidate substance in gluconeogenesis (Douce 1985). This observation may suggest that gluconeogenesis in seeds of canola is through glutamine-independent pathway.

Conclusions

Salt stress significantly decreased 100-seed weight and number of seeds per plant in canola. Also, the proteome of canola seeds changed at 350 mM NaCl. Generally, the abundance of proteins with antioxidant activity and storage proteins increased, while proteins involved in glycolytic and proteolytic activity showed downregulation. This study provided clues for basic insight needed for additional investigation on the response of canola seeds to salt stress at molecular level.

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تجزیه پروتئوم بذور کلزا برای شناسائی پروتئین‌های تغییر بیان یافته تحت تنش شوری

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چکیده

بذور همانند سایر قسمت‌های گیاه بخش مهمی هستند که توسط تنش شوری تحت تاثیر قرار می‌گیرند. درک سازوکارهای مولکولی که در بذور گیاه تحت تنش شوری اتفاق می‌افتد، از لحاظ توسعه کیفی اهمیت دارد. در این مطالعه، با استفاده از االکتروفورز ژل دو بعدی (2-DE) تلاش شده است تا پروتئین‌های با تغییرات بیان معنی‌دار بذور کلزا (رقم Hyola308) تحت تنش شوری شناسایی شود. نتایج نشان داد که تاثیر تنش شوری روی وزن صد دانه و تعداد دانه در سطح احتمال یک درصد معنی‌دار است و پروتئوم بذور متاثر از تنش می‌باشد. در کل از ۵۴۸ لکه پروتئینی تکرارپذیر، ۲۸ لکه پروتئینی تغییر بیان معنی‌دار نشان دادند که از آن‌ها هفت لکه دارای کاهش بیان بودند. آنالیز GO (هستی شناسی ژنی) پروتئین‌های با تغییرات بیان معنی‌دار را در شش فرآیند بیولوژیکی تقسیم بندی کرد: فرآیند اکسیداسیون-احیاء (۲۸٪/۵)، واکنش به تنش غیرزیستی (۲۸٪/۵)، واکنش به هورمون (۲۱٪/۴)، فرآیند کاتابولیکی (۲۱٪/۴)، فسفوریلاسیون دی فسفاتی نوکلئوزید (۱۷/۸٪) و فرآیند گلیکولیزی (۱۴٪/۲۸). در نهایت تنش شوری موجب تحریک بذور کلزا برای افزایش بیان پروتئین‌های دخیل در فعالیت آنتی اکسیدانی و پروتئین‌های با فعالیت ذخیره‌ای شد.

واژه‌های کلیدی: الکتروفورز ژل پلی‌آکریلامید دو بعدی؛ پروتئومیک؛ تنش غیرزیستی؛ فعالیت آنتی اکسیدانی؛ *Brassica napus*

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