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# Proteomic analysis of rapeseed (*Brassica napus L.*) seedling roots under salt stress

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## ABSTRACT

*Soil salinity is a major constraint in agricultural production. Although rapeseed is a salt tolerant plant, its oil production is reduced under salinity stress. To identify the mechanisms of salt responsiveness in rapeseed, the protein expression pattern of the roots in two contrasting cultivars were analyzed. Plants were exposed to 0, 175, and 350 mM sodium chloride. An increase in the sodium content and a reduction in growth and K content in both genotypes were observed under salt stress. The content of Na was more in the salt-sensitive compared with the other genotype particular in shoot. We applied a 2-DE based approach coupled with the identification of responsive proteins to analyze root samples. Out of 419 protein spots were detected, 20 and 21 proteins were differentially expressed in the susceptible and tolerant cultivars, respectively. Using MALDI TOF/TOF mass spectrometry analysis, 19 proteins could be identified. These spots had functions related to metabolism, transcription, translation, energy production, photosynthesis and electron transport. Results of this experiment suggest that these protein spots might play roles in adaptation to salinity stress. The roles of these proteins in rapeseed adaptation to salt stress will be discussed.*

**Keywords**: rapeseed, oxidative stress, proteome, salt stress.
***Abbreviations:*** ***DHAR****, dehydroascorbate reductase;* ***FBA****, fructose bisphosphate aldolase;* ***GR-RBP,*** *glycine-rich RNA-binding protein;* ***GST****, glutathione S-transferase;* ***HSP****, heat shock protein;* ***MLP****, Major latex protein;* ***NDPK****, nucleoside diphosphate kinase;* ***NTF-2****, nuclear transport factor 2;* ***ROS****, reactive oxygen species;* ***sHSP****, small heat shock protein;* ***SOD****, superoxide dismutase;* ***TPI****, Triose-phosphate isomerase;* ***Trxh****, thioredoxin h*

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## INTRODUCTION

Salt stress is a major abiotic stress in agriculture worldwide. It is estimated that about 20% of the earth's land mass and nearly half of all irrigated land are affected by salinity. Salinity causes water deficit and ion toxicity, leading to a decrease in biomass production. Salt stress leads to a secondary oxidative stress. Salinity can accelerate the production of reactive oxygen species (ROS) in cells [1]. Plants have mechanisms to protect them from the cytotoxic effects of ROS. These consist of osmolyte accumulation such as proline, as well as antioxidant enzymes [2].

Rapeseed (*Brassica napus L.*) is one of the oilseed crops being cultivated because of its high quality oil. Although *Brassica* species produce maximum yield under normal soil and environmental conditions, their production is

markedly reduced as a result of environmental stresses [3]. Rapeseed is sensitive to salt stress during the early growth stage [4], and this explains its classification as sensitive to salinity conditions at the mentioned stage [5].

Proteomics facilitates the comparison of proteins and provides knowledge about the proteins involved in plant responses. In this experiment, proteome analysis was performed on the roots of two canola cultivars, Sarigol (salt-sensitive ) and Hyola308 (salt-tolerant), to determine the differential expression of responsive proteins in roots, because the root is the organ of land plants most affected by salt stress. Proteins were separated by two-dimensional polyacrylamide gel electrophoresis and the differentially expressed protein spots were detected by mass spectrometry. The expression pattern of responsive proteins was analyzed using robust clustering methods.

## MATERIALS AND METHODS

*Plant materials and growth condition*

The experiment was conducted in hydroponic system. On the basis of previous studies [2,6] two rapeseed genotypes, Sarigol (salt sensitive) and Hyola308 (salt tolerant), were subjected to 0, 175, and 350 mM NaCl concentrations in a split plot design with three replicates. Three weeks after starting NaCl treatment, 45 plants were harvested for physiological analysis and proteomic work.

*Biomass and ion concentration*

Total dry weight and root dry weight was determined after drying the plant samples for 36 h at 75°C. K and Na content were assayed by flame photometer.

*Protein extraction and 2-DE*

Trichloroacetic acid (TCA)-acetone method [7] and phenol extraction method [8] were used for protein extraction. The difference in yield was visually noticeable on the 2-D patterns, with an increased number of spots using phenol extraction method as compared with TCA-acetone protocol, in both roots of rapeseed genotypes. So, phenol extraction method was used. Immobilized pH gradient strips (18 cm, pH 4–7, linear, BioRad) were loaded with sample proteins. Isoelectric focusing was conducted with a PROTEAN isoelectric focusing cell (BioRad). Immobilized pH gradient strips were applied using a PROTEAN II Multi Cell (BioRad). The spots in analytical and preparative gels were visualized by silver nitrate and Coomassie Brilliant Blue G-250 (CBB), respectively.

*Image analysis*

The analytical gels were scanned using GS-800 densitometer (BioRad) and analyzed using Melanie 7 software (GeneBio). The one-way ANOVA was carried out by SPSS program (version 20). Only those statistically significant protein spots ($P \leq 0.05$) were selected and they had to be consistently present in three replicates. The protein spots were filtered based on consistent and significant changes in both salt treatments and then expression level of two-fold.

*Protein classification and Identification*

Protein classification was performed on spots significantly affected by salt levels. Hierarchical clustering and self organizing map (SOM) methods were adapted using SPSS software version 20 and cluster software version 2.11 (http://rana.lbl.gov/EisenSoftware.htm).

Protein spots were identified using MALDI-TOF/ TOF MS analysis (Applied Biosystems 4700, USA) and Mascot search [9]. Combined MS-MS/MS searches were conducted with the selection of NCBInr database (Release 15.04.2009). The Probability score (95%) was used as criteria for identification.

## RESULTS

*Effect of salt stress on growth and ionic relations*

Salt levels had a statistically significant effect on all characteristics (data not shown). High salinity level had the greatest effect on dry matter reduction (Fig. 1a). Reduction of total dry weight was more pronounced in salt sensitive cultivar. Analysis of variance for the relative reduction in root matter showed different between two cultivars (data not shown). The relative reduction in root dry matter was the highest in susceptible cultivar, Sarigol (Fig. 1b). Under 350 mM NaCl salinity level, the root dry weight of sensitive cultivar was a fifth of the control dry weight, but in the tolerant cultivar was reduced to lower than of half that of the control.

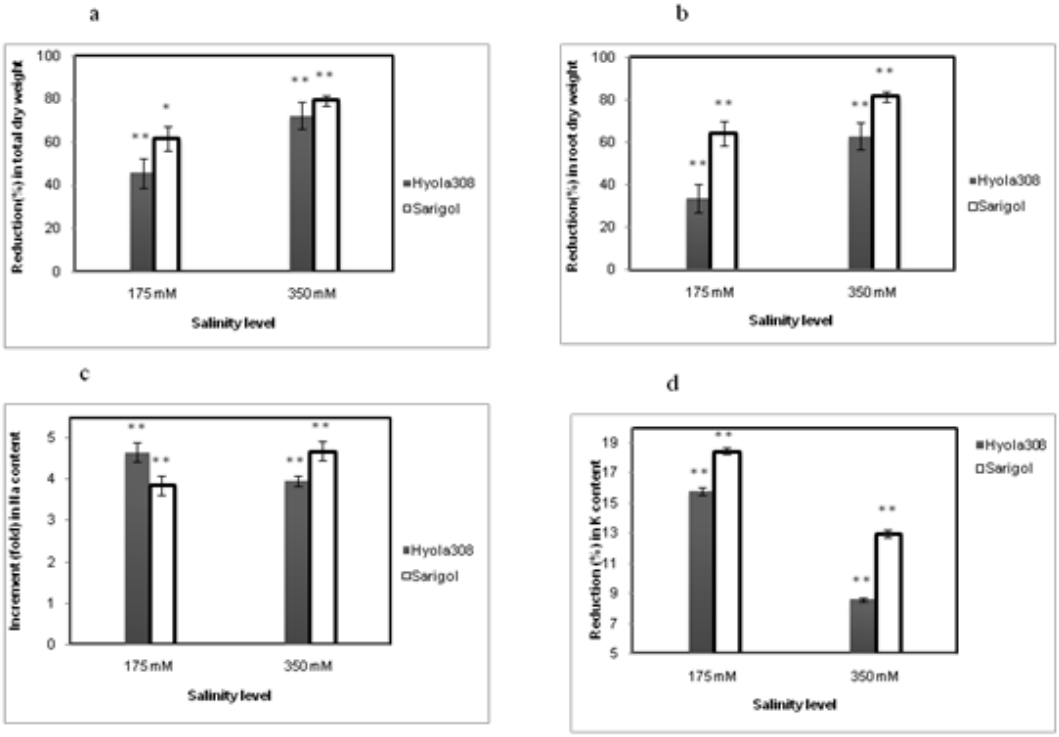


**Fig. 1. Effects of salinity treatments on reduction in total dry weight (a), root dry weight (b), increment in Na content (c) and reduction in K content (d) in roots of two canola cultivars. Plants were treated with 175 mM and 350 mM NaCl for 15 days. Means followed by * , ** and *** are significantly different (in comparison with control) at P<0.05, 0.01 and 0.001, respectively.**

The greatest effect of salinity on root ion concentrations was observed at 350 mM NaCl treatment (data not shown). Salinity stress increased the Na content of roots in two genotypes, but to a lesser extent in Hyola308 (Fig. 1c). K content was higher in roots of Hyola308 cultivar (Fig. 1d). Consequently, K/Na ratio in the roots of salt-stressed rapeseed plants was lesser than in control plants (data not shown). Increase in the Na content and reduction in K content was greatest in salt-stressed susceptible plants (Fig. 1c and Fig. 1d). In the presence of salt, Sarigol plants had a smaller K/Na ratio than Hyola308 plants. So, changes induced in protein spots in the roots of both genotypes were studied and the resulting protein expression patterns were quantitatively analyzed using image analysis package.

*Salt-responsive proteins*

Out of 419 spots reproducibly identified by 2-DE (Fig. 2), 41 were differentially expressed as a result of salinity stress. The number of differentially expressed proteins was 20 and 21 in susceptible and tolerant genotypes, respectively (Fig. 3). The number of induced protein spots (common/uncommon expressed in two genotypes) was 41 and 24 at high and low salinity levels, respectively. The greater part of differentially expressed spots were detected in Hyola308, where 7 proteins were downregulated and 14 proteins were upregulated, representing active proteome answer in this cultivar.

*Classification of proteins*

The salt-induced protein spot dataset was examined using SOM (nonhierarchical clustering method) and hierarchical clustering methods. Percent volume (Fig. 4a) and induction factor (Fig. 4b) data were used for clustering. The results of two types of clustering were parallel. Control treatment of the two cultivars placed in a distinct cluster. Root proteins of the salt-tolerant cultivar under both salinity treatments also placed in this group. Thus, Hyola308 is a constitutive genotype, and may explain Hyola308's tolerance. Protein profiles of Sarigol cultivar placed in a separated sub-cluster (Fig. 4a).

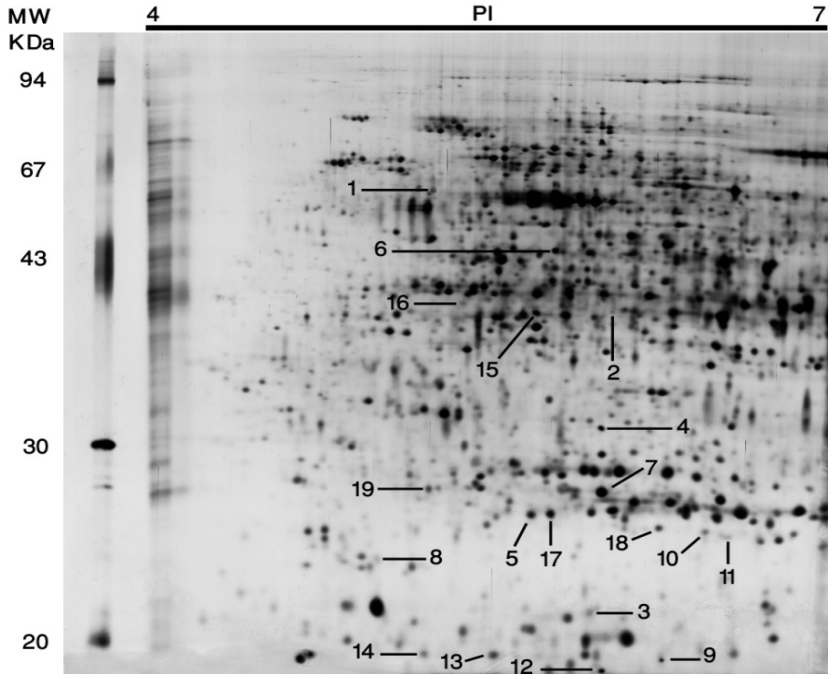


**Fig. 2. 2-D gel analysis of proteins extracted from roots of Hyola308 under 350 mM NaCl. Proteins were visualized by silver staining. Numbered spots correspond to salt-responsive proteins. Arrows indicate proteins analyzed by MS.**

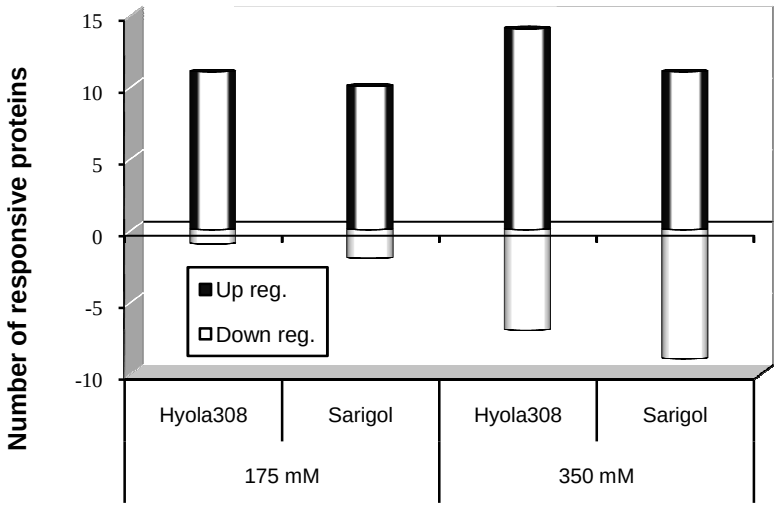


**Fig. 3. The numbers of up- and down-regulated proteins in roots of two canola genotypes at different salinity levels. Plants were treated with 175 and 350mM NaCl for 15 days. proteins were extracted from roots and separated by 2-DE.**

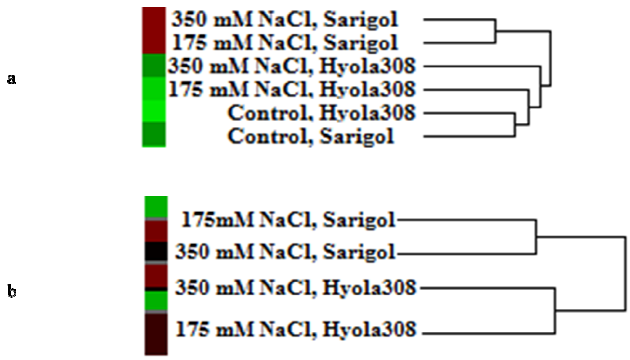


**Fig. 4. Hierarchical display of data from differential expression of protein spots under salt treatments. Hierarchical clustering analysis of the 41 differentially-responded proteins on the basis of (a) vol % and (b) induction factor.**

*Protein identification*

The MS results of 41 differentially detected spots resulted in the identification of 19 proteins (Table 1). The detected proteins were involved in oxidative stress, metabolism, transcription, translation, energy production, photosynthesis, regulating reactions and electron transport processes. The spots could be grouped to six clusters (Fig. 5). Five spots induced under oxidative stress were identified (spot 3, 4, 7, 12 and 17- Table 1). Furthermore, two spots (regulatory protein) were identified that involved in response to stress (spot 1 and 10). Protein involved in photosynthesis included the coproporphyrinogen III oxidase. Two spots, Triose-phosphate isomerase (TPI) and fructose bisphosphate aldolase (FBA) 2, involved in energy production. Two detected spots, thioredoxin-h-like protein 1 (Trxh-1) and putative nuclear transport factor 2 (NTF-2), contribute to electron transport and intracellular protein transport, respectively. In MS analysis also identified two spots involved in translation (spot 5) and transcription (spot 8), as well as nucleoside diphosphate kinase (NDPK) 1 (spot 11), contribute to nucleotide metabolism. Finally, we identified three small heat shock proteins (sHSPs) that were responded in rapeseed roots under stress conditions (spot 9, 18 and 19).

## DISCUSSION

Sarigol cultivar exhibited the maximum decline in root dry weight under salinity condition. The number of salt-induced protein spots was higher in Hyola308 (especially at 350 mM NaCl), suggesting active proteome response in Hyola308 as a tolerant genotype (Fig. 3). In the previous study on these two genotypes [2], the number of salt-induced proteins was maximum in leaves of Hyola308 cultivar. Active proteome answer of tolerant cultivar showed the capabilities of plant to exclude sodium ions and maintain grow. This genotype maintains the ionic stability and so its growth rate.

The number of induced protein spots detected following exposure to both salinity levels was 41 and 24, respectively, which may show the involvement of proteome behavior as plants adapt to elevated salinity condition. Under 175 mM NaCl salinity, both cultivars had a similar number of salt-induced protein spots. This trend has been reported by bandehhagh et al. [2,6] in which two cultivars had similar performance based on some characteristics under 175 mM NaCl level. These results show that high salt condition has determinant role in the extent of salt-induced protein expression.

**Table 1. Salt responsive proteins in roots of canola genotypes identified by MALDI-TOF/ TOF MS analysis and Mascot search**

| ID on gel | Homologous protein | Accession No.† | Exp. pI/MW (kDa) | Theo. pI/MW (kDa) | Protein Score | Cov (%) ‡ | MS-MS / PMF peptide |
|---|---|---|---|---|---|---|---|
| 1 | Chaperonin hsp60 | 16221 | 5.35 /64 | 5.66 / 61.3 | 124 | 10.57 | 2/4 |
| 2 | Coproporphyrinogen III oxidase, chloroplast (precursor) | 13431553 | 5.96 /39 | 6.24 / 43.8 | 424 | 25.91 | 7/9 |
| 3 | Cu/Zn superoxide dismutase | 63259317 | 5.89 /22 | 5.64 / 15.2 | 159 | 16.45 | 2/2 |
| 4 | Cu/Zn superoxide dismutase | 3273753 | 5.94 /31 | 6.28 / 22.2 | 93 | 16.67 | 2/3 |
| 5 | Eukaryotic translation initiation factor-5A | 40805177 | 5.63 /26 | 5.71 / 17 | 155 | 29.56 | 3/3 |
| 6 | Fructose bisphosphate aldolase 2 | 16226653 | 5.68/ 45 | 6.79/43 | 228 | 38 | 3/12 |
| 7 | Glutathione S-transferase 2 | 31790095 | 5.89 /28 | 5.66 / 24.3 | 646 | 63.85 | 7/12 |
| 8 | Glycine-rich RNA-binding protein 10 | 544416 | 5.12 /23 | 5.56 / 16.3 | 302 | 47.34 | 4/6 |
| 9 | HSP17.x | 8250122 | 6.30 /19 | 5.83 / 14.5 | 115 | 13.28 | 2/2 |
| 10 | Major latex protein-related / MLP-related | 18379240 | 6.42 /24 | 5.42 / 17.5 | 219 | 39.74 | 3/5 |
| 11 | Nucleoside diphosphate kinase 1 | 19570344 | 6.51 /24 | 6.30 / 16.4 | 313 | 39.86 | 5/5 |
| 12 | Putative dehydroascorbate reductase | 33285914 | 6.04 /18 | 6.15 / 12 | 223 | 40.74 | 3/4 |
| 13 | Putative nuclear transport factor 2 | 119720790 | 5.48 /20 | 5.69 / 13.6 | 123 | 11.38 | 2/2 |
| 14 | Thioredoxin-h-like protein 1 | 11494247 | 5.30 /20 | 5.35 / 12.9 | 150 | 29.31 | 2/3 |
| 15 | Triose-phosphate isomerase | 15226479 | 5.63 /39 | 7.67/33 | 249 | 51 | 2/14 |
| 16 | Triose-phosphate isomerase | 145329204 | 5.44 /40 | 7.02/32 | 209 | 44 | 2/12 |
| 17 | Type 2 peroxiredoxin | 4928472 | 5.67 /26 | 5.37/17 | 261 | 58 | 2/9 |
| 18 | 17.6 kDa class I small heat shock protein (HSP17.6B-CI) | 15227552 | 6.22 /25 | 6.33 / 17.6 | 133 | 20.92 | 2/5 |
| 19 | 22.0 kDa heat shock protein (ATHSP22.0) | 15234985 | 5.29 /28 | 5.58 / 22 | 207 | 22.05 | 3/4 |

*†) Accession number in NCBI.*

*‡) Percentage of the protein sequence covered by the matching peptides.*

**Table 1. Continued**

| ID on Gel | Species | Expression level † | | | |
|---|---|---|---|---|---|
| | | Sarigol IF1‡ | Sarigol IF2 | Hyola308 IF1 | Hyola308 IF2 |
| 1 | *Arabidopsis thaliana* | -1.4 a | -2.5 c | -1.4 a | -1.9 b |
| 2 | *Arabidopsis thaliana* | -1.8 b | -2.3 bc | -1.7 a | -2.8 c |
| 3 | *Brassica napus* | -4.1 b | -4.7 a | 2.0 d | 2.5 c |
| 4 | *Arabidopsis thaliana* | 1.6 d | 5.9 b | 3.4 c | 14.2 a |
| 5 | *Brassica napus* | 1.5 d | 7.5 b | 5.4 c | 15.1 a |
| 6 | *Arabidopsis thaliana* | 1.1 bc | 3.1 b | 1.3 c | 6.2 a |
| 7 | *Brassica juncea* | 2.6 d | 3.9 c | 3.4 b | 5.9 a |
| 8 | *Brassica napus* | -1.6 b | -2.9 c | -1.3 a | -1.5 b |
| 9 | *Brassica oleracea var. alboglabra* | 1.6 d | 1.9 c | 2.4 b | 3.9 a |
| 10 | *Arabidopsis thaliana* | -1.7 a | -2.9 c | -1.7 a | -2.3 b |
| 11 | *Brassica rapa* | -1.7 a | -4.3 c | -1.6 a | -3.1 b |
| 12 | *Brassica rapa* | 1.6 d | 2.6 b | 2.4 c | 2.9 a |
| 13 | *Brassica rapa* | 1.8 b | 2.2 a | 1.2 c | 1.4 c |
| 14 | *Brassica oleracea* | Presented only in salinity levels, with higher expression in Hyola308 | | | |
| 15 | *Arabidopsis thaliana* | 2.3 bc | 2.1 c | 3.1 b | 5.5 a |
| 16 | *Arabidopsis thaliana* | -1.6 c | -3.4 c | -1.6 a | -1.9 b |
| 17 | *Brassica rapa subsp. pekinensis* | 1.6 c | 2.8 b | 1.8 c | 5.3 a |
| 18 | *Arabidopsis thaliana* | Presented only in salinity levels, with higher expression in Sarigol | | | |
| 19 | *Arabidopsis thaliana* | 1.9 c | 2.1 c | 3.2 b | 4.1 a |

*†) Means followed by the same letter in a group are not significantly different at P<0.05.*

*‡) IF1 and IF2 are the induction factors (%volume of protein in stress condition / %volume of protein in stress condition) at 175 and 350mM NaCl, respectively.*

Plant may possibly employ ROS as signaling molecules for increasing oxidative stress enzymes production during acclimation to high salinity stress. ROS are highly reactive, and can seriously disturb usual metabolism through oxidative damage to proteome [10, 11]. SOD is a main scavenger of $O_2^-$. Activity of this enzyme results in the formation of $H_2O_2$. Type 2 peroxiredoxin catalyzes the breakdown of $H_2O_2$. GSTs and DHARs are involved in detoxification and protection against oxidative stress via scavenging of ROS. In this experiment, salt condition resulted in decrease in abundance of SOD (spot 3) in sensitive cultivar and an increase in tolerant cultivar. Equilibrium between abundance of ROS and the quenching action of antioxidant enzymes is disturb under salinity treatments, and the extent of imbalance shows the rate of sensitivity to salinity [12]. The abundance of another isoform of SOD (spot 4) increased in both genotypes with higher expression in Hyola308 genotype. This accumulation has protective function, and has been reported in rapeseed leaves [2], rice [13] and sugar beets [14]

in response to salinity and drought stresses. Type 2 peroxiredoxin (spot 17) was expressed in both cultivars, but the increase in abundance was the highest in tolerant cultivar, suggesting that type 2 peroxiredoxin may be responsible for the elevated tolerance. Spot 17 (with the same accession number) detected only in the leaf 2 of these two genotypes under the same salinity levels [2]. Leaf 2 had a discrimination task between both cultivars under salinity levels. GST- 2 (spot 7) and DHAR (spot 12) up-regulated in response to salinity. However, increase in abundance was elevated in Hyola308 compared with susceptible cultivar. The up-regulation of GST- 2 and DHAR under salinity has been reported by Sugimoto and Takeda [15].

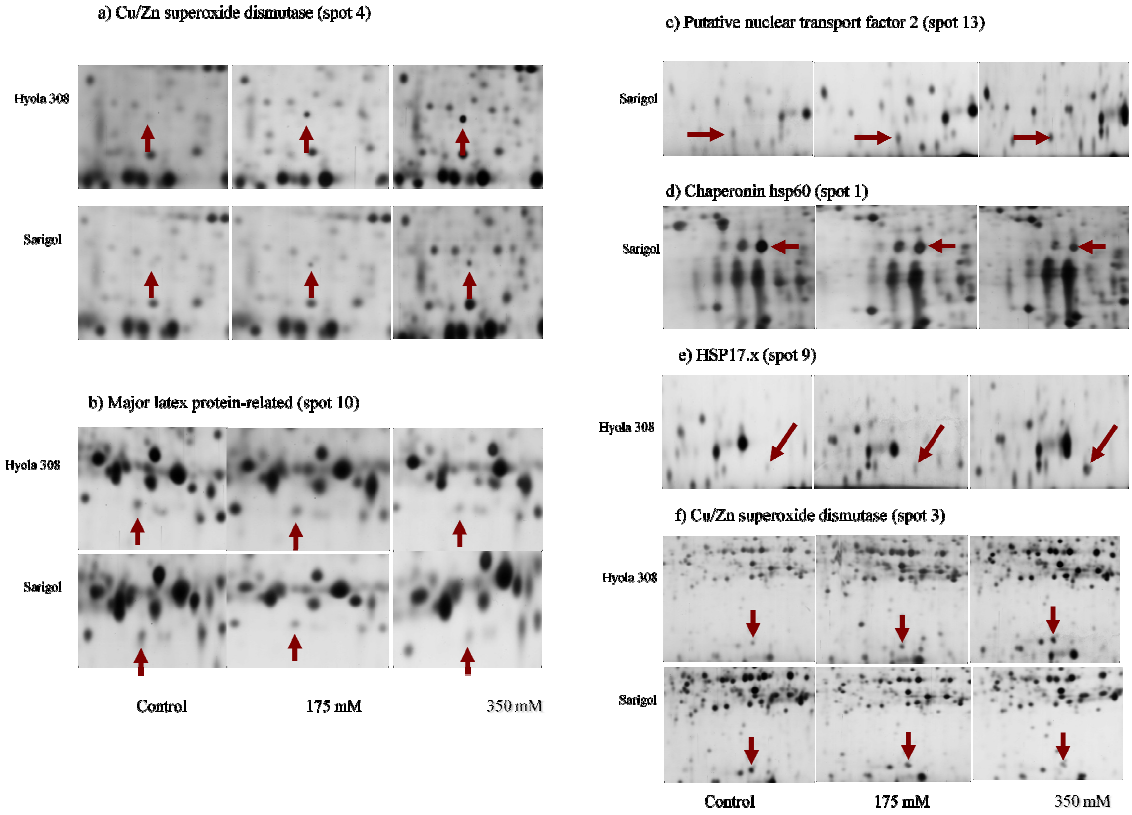


**Fig. 5. Expression pattern of salt-responsive proteins in roots of canola genotypes at 0, 175 and 350 mM NaCl. Responsive proteins could be clustered to six classes. a) Cu/Zn superoxide dismutase (spot 4), up-regulated in both genotypes; b) Major latex protein-related (spot 10), down-regulated in both genotypes; c) Putative nuclear transport factor 2 (spot 13), up-regulated in sensitive genotype; d) Chaperonin hsp60 (spot 1), down-regulated in sensitive genotype; e) HSP17.x (spot 9), up-regulated in tolerant genotype; and f) Cu/Zn superoxide dismutase (spot 3), up-regulated in the tolerant genotype and down-regulated in the sensitive genotype.**

Major latex protein (MLP)-related (spot 10) and chaperonin hsp60 (spot 1), regulatory protein, down-regulated in response to salinity. The decrease in abundance was greater in sensitive compared with tolerant genotype. The down-regulation of these two regulatory proteins under salinity stress has not been reported previously. HSP60 is a mitochondrial chaperonin that is typically held responsible for the transportation and refolding of proteins from the cytoplasm into the mitochondrial matrix. In addition to its role as a heat shock protein, HSP60 functions as a chaperonin to assist in folding linear amino acid chains into their respective three-dimensional structure. While the function of the MLPs is unknown, they have been associated with flower development and in pathogen defense responses [16].

The photosynthesis-associated protein coproporphyrinogen III oxidase (spot 2) catalyzes the oxidative decarboxylation of coproporphyrinogen III to proto-porhyrinogen IX in the haem and chlorophyll biosynthetic pathways. We observed down-regulation of this spot in two cultivars. However, the most decrease in abundance

was observed in Hyola308 cultivar. This protein had similar expression in the leaves of these genotypes under salinity stress [2]. The down-regulation of this protein under salt stress has reported in *Thellungiella halophila* and *Arabidopsis thaliana* [17].

Increased abundance of spot 15 (one isoform of TPI) was higher in Hyola308 compared with susceptible cultivar. Decreased abundance of spot 16 (another isoform of TPI) was higher in sensitive compared with tolerant genotype. The up- and down-regulation of spot 15 and 16 (with the same accession number) have occurred in the leaves of Sarigol and Hyola308 under the same salinity levels [2]. It may be possible that the increase and decrease of two spots of TPI (with similar Mw and pI) represent the possibility of translation modification like phosphorylation. The up-regulation of TPI in rice under salinity stress has been reported by Dooki et al. [18]. The high expression of TPI in tolerant genotype is necessary, because it is essential for ATP production and its increased abundance reflects changed patterns of carbon flux in response to low level of photosynthesis. The other energy production-associated protein FBA 2 (spot 6) catalyzes the cleavage of fructose 1, 6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (DHAP). In this experiment FBA 2 was up-regulated under salinity stress in both rapeseed, but with higher expression in tolerant genotype. Two isoforms of this protein had oppositional expression in the leaves of these two rapeseed genotypes [2]. One isoform showed up-regulation in response to salinity in both rapeseed and other isoform down-regulated. Totally, the abundance of FBA was the highest in tolerant genotype. FBA decreased in intensity in response to salinity in roots of cucumber seedling [19]. In contrast, in rice leaf sheath exposed to NaCl, FBA showed up-regulation in abundance [13]. In this study, up-regulation of FBA might have changed the levels of sugars and starch and might have stimulated growth rate of rapeseed roots under salinity, especially in tolerant genotype.

Two identified proteins, Trxh-1(spot 14) and NTF-2 (spot 13), contribute to electron transport and intracellular protein transport, respectively. Thioredoxins are small proteins participating in numerous dithiol/disulfide interchange reactions. One of the established functions of thioredoxins is to reduce disulfide bonds in target proteins. The thioredoxins are catagorized depending on localisation in the cell and cytosolic thioredoxins are classified as thioredoxin h. Thioredoxin act as a major defence system against oxidative damage by reducing the disulphide bonds of oxidized proteins. In this experiment Trxh-1 presented only in salinity levels, with higher expression in Hyola308. Such a pattern of variations suggested that, accumulation of Trxh-1in under salinity treatment has a protective role in tolerant genotype.

The main role of NTF-2 is to transport RanGDP from cytoplasm to nucleus by interacting with FxFG nucleoporin repeats. Ran is a GTP binding protein that is essential for the translocation of RNA and proteins. NTF-2 showed up-regulation only in susceptible genotype under high salinity.

Three small heat shock proteins (sHSPs, spot 9, 18 and 19) were induced in rapeseed roots under high salinity level. Additionally to heat stress, sHSPs are expressed in palnt tissues in response to drought stress [20]. The sHSPs decrease the level of ROS, thereby protecting photosystem II reaction during stress [21]. An HSP protein spot was detected by proteome study of wild watermelon [22] and sugar beet leaves [14] under drought stress. In our study, tow sHSPs (spot 9 and 19) up-regulated in response to stress with higher abundance in tolerant genotype. Other sHSP (spot 18) presented only in salinity levels. These findings are consistent with reports in alga (*Dunaliella salina*; [23]) and rice [24, 25]. Thus, during adaptation to salinity condition, the HSP family might play a vital task in the regulation of root growth and development of rapeseed plants.

Spot 5 (eukaryotic translation initiation factor-5A) up-regulated in both genotypes, but with higher abundance in tolerant genotype in response to salinity. This spot was detected only in leaves of tolerant rapeseed genotype under salinity stress [2]. This factor involves in the primary step of peptide bond formation through translation process, and to be involved in regulation of cell-cycle and also in RNA binding [26]. In a report [27] this protein spot was restricted in a cultivar of wheat (Jinan 177) but decreased in an introgression strain of *Triticum aestivum*/*Thinopyrum ponticum* in response to salt treatments. A decline in expression of eukaryotic translation initiation factor-5A is a sign of cell senescence in response to salinity condition due to change in the cell cycle.

Glycine-rich RNA-binding protein 10 (GR-RBP 10; spot 8) has a role in RNA transcription or processing during stress. The expression of protein spot 8 was reduced in Sarigol plants under salinity treatments. GR-RBPs play certain roles in post-transcriptional regulation of gene expression in plants under various stress conditions [28].

They may play important roles in stress responses, as their mRNA levels increased after exposure to water stress [29]. This may explain one of the several reasons of Hyola308's tolerance.

Spot 11 matched to NDPK 1 that decreased up to 4.3 fold under stress in the susceptible genotype. The up-regulation of this protein has been reported under drought stress [7,14] and salt stresses [30]. NDPK is a housekeeping gene. This protein uses ATP to maintain cellular levels of CTP, GTP, and UTP. Over expression of NDPK in Arabidopsis plant down-regulated the accumulation of ROS and enhanced the tolerance to abiotic stresses [31]. In this experiment NDPK 1 down-regulated under stress and decrease in abundance was greater in sensitive compared with tolerant cultivar. Down-regulation of NDPK in response to salinity has not been reported previously.

In this work, we identified a number of salt-induced protein spots in the roots of tolerant cultivar, which maintains growth rate during stress. Roots of tolerant genotype expressed more responsive proteins than susceptible cultivar. Hyola308 could therefore tolerate stress condition better than Sarigol, with a lesser amount of a reduction in biomass production. Cluster analysis based on responsive proteins indicated that Hyola308 is a constitutive tolerant accession, and may explain this Hyola308's tolerance. A major fraction of detected protein spots involved in oxidative stress responses and then energy production. These findings suggest that oxidative stress-related proteins have a vital task in the adaptation of rapeseed roots to elevated salinity condition.

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