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Original Scientific Paper

Alterations in the root proteomes of *Brassica napus* cultivars under salt stress

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ABSTRACT:
Soil salinization is an important environmental problem affecting agricultural production worldwide. Seed germination is a critical process, and seedling establishment under saline conditions can be achieved by successful germination. In the present study, comparative proteomics combined with physiological analyses were used to investigate the protein alterations in germinating *Brassica napus* cultivars (Caravel and Sary) under NaCl stress. Seed germination declined with the increasing NaCl concentration. However, Caravel exhibited better performance in terms of seed germination and seedling growth under salinity stress. Therefore, Caravel was found to be more tolerant to salinity than Sary. The root proteins were extracted from *B. napus* cultivars germinating on a plant growth medium with or without 100 mM NaCl for seven days. After the root proteins had been separated by two-dimensional (2-D) gel electrophoresis, the differentially accumulated proteins were identified using MALDI-TOF/TOF MS. The comparative proteomics analysis revealed 12 and 27 statistically significant proteins accumulated in the NaCl-treated roots of Caravel and Sary, respectively. The identified proteins were mostly involved in protein metabolism, stress defense, cytoskeleton and cell wall metabolism, and energy metabolism. The salt-sensitive cultivar Sary displayed an elevated accumulation of proteins involved in antioxidant defense and the protein catabolic process such as superoxide dismutase [Fe], L-ascorbate peroxidase 1, and different components of the proteasome system. On the other hand, the levels of molecular chaperones including 20 kDa chaperonin, chaperonin CPN60, heat shock cognate protein HSC70, and heat shock 70 kDa protein 1 were higher in Caravel than Sary under salt stress. These findings will provide the possible mechanisms which contribute to salt tolerance and may serve as the basis for improving salinity tolerance in rapeseed.

Keywords:
Brassica napus, germination, proteomics, salt tolerance

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INTRODUCTION

Soil salinity is one of the major environmental factors limiting crop production. Excessive salinity suppresses plant growth by inducing ion toxicity, osmotic stress, and oxidative damage (Munns & Gilliham 2015). Considering the increased demand for food for the growing global human population, the development of crops tolerant to salinity has become a priority for global breeding programmes. Numerous studies have attempted to explain the mechanism of salinity tolerance and provided information on salt-responsive genes and proteins in plants (Singh *et al.* 2017; Wang *et al.* 2019; Zhang *et al.* 2019). To cope with salinity stress, plants have evolved a number of adaptive mechanisms including the adjustment of ion homeostasis, the synthesis of compatible solutes, the regulation of hormones, the induction of antioxidant enzymes, and the modulation of signaling pathways (Zhang *et al.* 2012).

A plant's tolerance to salinity is affected by not only

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the degree of stress, but also its developmental stage (Elias *et al.* 2020). Seed germination is a complex physiological process in the plant life cycle and is influenced by environmental factors (Llanes *et al.* 2016). Salt-induced inhibition in seed germination has been well documented in many crop species including *B. napus* (Fang *et al.* 2017; Tan *et al.* 2017). Although a large number of studies have focused on investigating the responses of different plant species to salinity (Jia *et al.* 2015; Wang *et al.* 2019; Zhang *et al.* 2019), proteomic studies at the germination stage have been limited. It is well documented that the adaptation to salinity stress requires alteration in gene expression thus leading to changes in the protein profile (Aghaei & Komatsu 2013). The identification of genes or proteins conferring salinity tolerance will be helpful in gaining an understanding of the molecular mechanisms and breeding of salt-tolerant cultivars.

Proteomics is an effective tool to address the biological function of proteins in response to abiotic stress including salinity stress (Hernández *et al.* 2017). Comparative proteomics can identify the specific proteins which are regulated by salinity during seed germination. Debez *et al.* (2012) reported that salinity regulated sixty-seven proteins during seed germination in *Cakile maritima*, most of which were involved in storage, energy metabolism, primary metabolism, and stress defense. Yin *et al.* (2015) suggested that exogenous calcium helped soybeans to combat salinity stress by enriching signal transduction, promoting protein biosynthesis, energy pathway and transportation, regulating protein processing, enriching antioxidant enzymes, and redistributing storage proteins. Another proteomic study demonstrated that exogenous melatonin promoted the stress defense and energy metabolism in germinating cucumber seeds under salinity stress (Zhang *et al.* 2017). However, only a few studies have examined the effects of salinity on the proteome alterations during seed germination.

Rapeseed (*Brassica napus* L.) is one of the important sources of edible oils in the world. However, its yield is negatively affected by abiotic environmental stresses such as salinity stress (Shabani *et al.* 2015). To the best of our knowledge, studies comparing the salt-response proteomes in *B. napus* cultivars with contrasting salt tolerance have not been carried out. In the present study, a 2-D-based proteomic approach coupled with MALDI-TOF/TOF MS was performed to examine proteome alterations in the roots of rapeseed cultivars in the early germination stage. The aim of our study was to identify some of the salt-responsive proteins and pathways involved in the adaptation of germinating rapeseed cultivars to salinity stress as well as to provide insight into the molecular mechanisms underlying salinity tolerance.

MATERIALS AND METHODS

Seed germination experiments. Fifty seeds of rapeseed (*B. napus*) cultivars (Caravel and Sary) were placed in petri dishes (10 cm diameter) containing filter paper moistened with distilled water (control) or NaCl solutions in a wide range of concentrations (100, 150, 200, 250, 300, 350 and 400 mM). The petri dishes were covered with parafilm to avoid evaporation. The seeds were maintained in the growth chamber in darkness at 25°C for five days. Three replicates were conducted for each treatment. A seed was considered as having germinated when the radicle was longer than 2 mm.

Seedling growth and proteomic analysis. In order to evaluate the effects of salinity stress on seedling growth and root proteome, 100 mM NaCl was used. Preliminary experiments with different NaCl concentrations (0, 100, 200, and 300 mM) were carried out to determine the appropriate test concentrations. The NaCl concentration was limited to 100 mM because the seedlings suffered too much toxicity and damage at 200 mM NaCl and above. The seeds were placed on a plant growth basal medium (Murashige and Skoog, MS) solidified with 0.8% (w/v) agar in Magenta vessels. The MS basal medium was supplemented with either 100 mM NaCl or without NaCl (control). The cultures vessels were maintained at 25°C under a 16 h/8 h (day/night) photoperiod with 250 μmol m^{-2} s^{-1} photosynthetically active radiation for seven days. At least twenty seedlings from each replicate were separated into the roots and the shoots (hypocotyls+cotyledons). After the determination of the fresh weight (FW), the dry weight (DW) was detected by the incubation of the plant tissues at 80°C for 48 h. For proteomic analysis, the fresh root tissues were frozen in liquid nitrogen and stored at –80°C.

Protein extraction and 2D gel electrophoresis. Root proteins from three biological replicates were prepared according to the phenol extraction method described by Ahsan *et al.* (2008). The root samples (1 g) were powdered in liquid nitrogen and suspended in ten volumes of extraction buffer containing 20 mM $MgCl_2$, 0.5 M Tris–HCl (pH 8.3), 2% (v/v) β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 2% (v/v) NP-40, and 0.7 M sucrose. The homogenates were then mixed with an equal volume of Tris–HCl-saturated phenol (pH 8.0). After centrifugation, the proteins in the upper phenol phase were precipitated using four volumes of 100 mM ammonium acetate in methanol. Following precipitation overnight at –20°C, the proteins were obtained by centrifugation and washed three times with 100 mM ammonium acetate in methanol. The protein pellet was finally vacuum-dried and dissolved in lysis solution containing 7 M urea, 2 M thiourea, 65 mM dithiothreitol (DTT), 1 mM PMSF 4% CHAPS, and 0.2% v/v ampholy-

tes. The protein concentration was quantified using the Bradford method (Bradford 1976).

In order to separate the root proteins of the rapeseed cultivars, isoelectric focusing (IEF) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were carried out. For the analytical gels used in the image analysis, immobilized pH gradient (IPG) strips were treated with a rehydration buffer containing 80 µg proteins, while 500 µg proteins were used for the preparative gels in the mass spectrometry analysis. After passive rehydration for 16 hours in laboratory conditions, IEF was performed at 20°C using a Protean® i12™ IEF System (Bio-Rad, USA) with the following settings: 250 V for 30 min, ramping to 10000 V for 2 h, and maintaining the voltage at 10000 V for a total of 80000 V h. The resulting strips were first treated with an equilibration solution (6 M urea, 50 mM Tris-HCl buffer pH 8.8, 20% glycerol, and 2% SDS) containing 1% DTT, and then with an equilibration solution containing 2.5% iodoacetamide for another 15 min. The SDS-PAGE was carried out with 12.5% polyacrylamide gels using Protean II XL Cell (Bio-Rad). The gels were run at 16 mA per gel for 30 min, followed by 24 mA per gel for 5 h. The proteins resolved on the analytical gels were detected by silver staining (Sinha *et al.* 2001) while those on the preparative gels were stained with blue-silver staining (Candiano *et al.* 2004).

Image analysis, in-gel digestion, and mass spectrometry. Images of the triplicate silver stained gels were obtained using the ChemiDoc MP System (Bio-Rad). Spot detection and matching were carried out using PDQuest software ver. 8.0.1 (Bio-Rad). The intensity of each spot was normalized to the total density of all valid spots to compensate for any differences in gel staining. The proteins which exhibited at least a 1.5-fold change between the control and the NaCl treatment were considered as differentially expressed proteins ($P < 0.05$ by Student's t-test). The protein spots were manually excised from the preparative gels and digested with trypsin via a commercial kit (Thermo Fisher Scientific).

A solution of digested peptides was purified using ZipTip C_{18} pipette tips (Millipore), and mixed with the matrix solution (α-cyano-hydroxy cinnamic acid), and then spotted onto a MALDI plate. Peptide mass spectra were obtained with AB Sciex TOF/TOF 5800 mass spectrometer (Applied Biosystems). The obtained MS/MS spectra for each spot were submitted to the MASCOT search engine (http://www.matrixscience.com). The parameters for the search were as follows: taxonomy, Viridiplantae (green plants); database, Swiss-Prot; enzyme, trypsin; mass accuracy, 50 ppm; peptide MS tolerance, ±0.4 Da; variable modifications, oxidation of methionine; fixed modification, carbamidomethylation of cysteine; and the allowance of one missed cleavage. The differentially expressed proteins were classified on the basis of functions according to the database at https://www.uniprot.org/uniprot/.

Statistical analysis. The statistical analyses were performed by analysis of variance (ANOVA) using SPSS 22.0 software. The mean values from the physiological parameters were statistically analyzed with Duncan's multiple range test (DMRT). The differences in protein abundance were analyzed using Student's t tests, and were considered as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Seed germination and seedling growth under NaCl stress. Germination is one of the effective criteria used for the assessment of salt tolerance in plants. In the present study, the salt tolerance of two rapeseed cultivars was evaluated at the stages of seed germination and early seedling growth. The results of the present study showed that the germination percentage of both rapeseed cultivars was negatively affected by the increasing NaCl concentration (Fig. 1). Our results also indicated that there was a significant difference between the germination of the two cultivars. At 200, 250, 300, and 350 mM NaCl concentrations, the final germination of Sary was lower than that of Caravel. However, no germination was observed for salinities above 250 mM NaCl in Sary, whereas the germination of Caravel was completely inhibited at 400 mM NaCl. In our study, Caravel was identified as relatively salt tolerant, while Sary was identified as a relatively salt sensitive cultivar. Nichols *et al.* (2008) suggested that the adaptation of plants to salinity depends on genotype-specific salt-tolerance mechanisms.

Although rapeseed is considered a salt-tolerant oilseed crop (Ashraf & McNeilly 2004), salinity stress causes a reduction in growth parameters. In the present study, 100 mM NaCl was used to evaluate the salt tolerance of rapeseed cultivars at the early seedling stage. Salinity stress significantly decreased the fresh weight of both cultivars, but this effect was more pronounced in Sary (Fig. 2). A similar trend was also observed for the root dry weight. On the other hand, salt stress resulted in an increase in the dry weight of the shoots of Caravel. According to Wu *et al.* (2019), the shoot fresh weight can be used as a credible criterion for estimating the salinity tolerance of rapeseed genotypes. The fresh weight of the shoots in the Caravel and Sary cultivars were reduced by about 9.7% and 40.4%, respectively. These results show that Caravel is relatively tolerant to salt and Sary is relatively sensitive, which is in accordance with the germination responses.

Proteomic alterations under NaCl stress. In order to improve salt tolerance in rapeseed, it is necessary to understand the salinity tolerance mechanisms in the germination and early growth stages. Since these stages

Table 1. List of differentially accumulated proteins in the roots of the rapeseed cultivars exposed to NaCl stress

Spot	Accession number	Protein	Score	Exp./ Teo. Mw/pI	MP	Seq. Cover.	Protein expression	
							Caravel (NaCl *vs* C)	Sary (NaCl *vs* C)
Protein metabolism								
8	XP_013720288.1	Proteasome subunit beta type-6	145	E:27.3/5.72 T:25.1/5.18	12	66%	1.00	+1.70
10	XP_013722011.1	20 kDa chaperonin	325	E:28.0/5.61 T:26.7/8.57	26	66%	+1.54	0.84
13	XP_013698990.1	Proteasome subunit alpha type-2-A-like	98	E:29.6/5.62 T:25.8/5.38	12	60%	0.81	+1.59
14	XP_013724873.1	Proteasome subunit beta type-7-A-like	166	E:29.6/6.58 T:29.5/7.08	19	51%	0.74	+1.56
17	XP_013676342.1	PYK10-binding protein 2-like	329	E:34.9/6.09 T:32.0/5.73	24	85%	1.05	+1.53
26	XP_013672973.1	26S protease regulatory subunit 7 homolog A-like	361	E:52.6/6.51 T:47.8/6.33	33	54%	1.07	+1.62
29	XP_013725105.1	PYK10-binding protein 1-like	159	E:59.9/5.28 T:48.1/4.98	23	67%	+2.25	0.92
30	XP_013731995.1	Probable mitochondrial-processing peptidase subunit beta	222	E:61.0/6.21 T:59.0/6.14	23	36%	1.11	+1.73
31	XP_013711535.1	Chaperonin CPN60, mitochondrial	226	E:63.3/5.49 T:61.3/5.64	21	30%	+2.51	1.14
34	gb|AAB88009.1	Heat shock cognate protein HSC70	403	E:76.8/5.26 T:70.7/5.08	40	48%	+2.32	0.97
35	XP_013655629.1	Heat shock 70 kDa protein 1	194	E:81.8/5.21 T:71.0/5.05	24	24%	1.01	−1.61
Stress response								
2	gb|AID60095.1	CAP superfamily protein	58	E:16.0/6.21 T:19.4/6.06	4	20%	+1,66	0,91
3	XP_013692704.1	Universal stress protein YxiE-like	228	E:18.5/6.49 T:17.9/6.06	16	63%	1,22	+1,95
4	XP_013738491.1	Peroxiredoxin-2B-like	94	E:22.1/5.65 T:17.4/5.39	9	70%	0,84	+1,59
7	XP_013641388.1	Superoxide dismutase [Fe]	88	E:26.6/6.14 T:23.8/6.16	6	14%	1.34	+3.05
9	XP_013693377.1	NAD(P)H dehydrogenase (quinone) FQR1-like	301	E:27.3/6.54 T:21.8/6.43	20	55%	1.07	+1.62
11	XP_013722742.1	Glutathione S-transferase F3-like	501	E:28.0/5.92 T:24.3/5.66	25	77%	0.84	+1.70
12	XP_013698693.1	Glutathione S-transferase U27-like	272	E:28.6/6.08 T:26.7/5.83	17	30%	+1.84	−1.98
15	XP_013716521.1	L-ascorbate peroxidase 1	90	E:30.2/6.15 T:27.5/5.73	15	58%	1.04	+2.00
25	XP_013724800.1	Probable monodehydroascorbate reductase, cytoplasmic isoform 4	527	E:48.6/5.24 T:47.0/5.09	35	72%	−1.50	1.18
Energy and carbohydrate metabolism								
5	XP_013663145.1	ATP synthase subunit d, mitochondrial	125	E:22.3/4.94 T:19.6/4.92	14	53%	1.08	−1.72
20	XP_013748997.1	Malate dehydrogenase 1, mitochondrial-like isoform X1	366	E:37.0/6.41 T:35.7/8.81	19	42%	−1.85	0.83
21	XP_013748848.1	Malate dehydrogenase 1, mitochondrial-like	195	E:39.0/6.19 T:35.9/8.81	12	11%	0.91	−1.59
32	XP_013696878.1	2,3-bisphosphoglycerate-independent phosphoglycerate mutase 2	423	E:68.5/5.75 T:61.0/5.49	37	61%	1.05	−2.71
36	XP_013741984.1	Transketolase-1	169	E:85.2/5.75 T:79.2/5.96	19	35%	1.07	−2.00

Cytoskeleton and cell wall								
1	XP_013732486.1	Profilin-1-like	68	E:14.4/4.66 T:14.0/4.56	9	51%	1.39	+1.60
27	XP_013720668.1	Tubulin alpha-6 chain	205	E:55.7/5.25 T:49.5/4.93	23	47%	1.16	+3.64
28	XP_013685704.1	Tubulin beta-4 chain-like	533	E:57.3/5.09 T:49.8/4.72	38	58%	+1.59	1.02
33	XP_013724063.1	Beta-glucosidase 23	216	E:68.8/6.66 T:60.0/6.36	24	45%	0.92	+2.13
Signal transduction								
6	XP_013653355.1	Translationally-controlled tumor protein homolog	142	E:25.5/4.78 T:19.0/4.57	10	58%	+1.73	1.03
19	XP_013729792.1	Annexin D2	644	E:36.9/6.36 T:36.0/5.97	40	73%	−1.61	1.16
Nitrogen metabolism								
23	XP_013683139.1	Glutamate dehydrogenase 1	308	E:45.0/6.52 T:44.6/6.10	23	49%	+1.83	+1.96
24	XP_013701876.1	Glutamate dehydrogenase 2-like	358	E:45.2/6.36 T:44.6/5.93	25	53%	1.12	+2.51
Lipid metabolism								
22	emb|CAA65990.1	Acyl-[acyl-carrier protein] desaturase	377	E:53.2/5.71 T:45.3/5.75	31	65%	1.18	+1.62
Others								
16	XP_013734824.1	Phosphomannomutase	248	E:30.8/5.57 T:27.9/5.37	17	50%	0.94	−1.67
18	emb|CDX82402.1	BnaA03g34190D	222	E:36.2/5.36 T:32.0/5.16	22	65%	0.90	+2.00
37	XP_013684600.1	Cell division control protein 48 homolog A-like	69	E:97.4/5.37 T:89.4/5.10	19	30%	1.15	−2.38

are more vulnerable to salinity stress, molecular studies could be useful for developing tolerant cultivars. Therefore, the major aim of this research was to reveal the salt-induced proteomic alterations in the roots of rapeseed cultivars at the early growth stage. To determine the proteome alterations, IEF/SDS-PAGE and MALDI-TOF/TOF mass spectrometry were used. A total of 39 proteins were found with a more than 1.5-fold change in the germinating rapeseed roots, and 37 proteins were successfully identified by the MS analysis (Fig. 3). The list of the identified proteins is given in Table 1. Among them, one protein was up-regulated by NaCl in both cultivars. Six proteins were down-regulated in Sary, with no significant change observed in the Caravel cultivar. In contrast, 3 proteins were down-regulated in Caravel, with no significant change noted in Sary. A total of 9 and 19 proteins were significantly increased in Caravel and Sary, respectively.

The identified proteins in the roots of both cultivars were classified into various functional groups including protein metabolism (29.7%), stress defense (24.3%), energy and carbohydrate metabolism (13.5%), cytoskeleton and cell wall metabolism (10.8%), signal transduction (5.4%), nitrogen metabolism (5.4%), lipid metabolism (2.7%), and others (8.1%) (Table 1). In general, most of the proteins related to defense and protein degradation showed an up-regulation in the Sary cultivar, whereas most of the up-regulated proteins in Caravel were identified as molecular chaperons. The differential regulation of these proteins under NaCl stress is discussed below.

Salinity stress may negatively affect cellular homoeostasis by leading to metabolic disruption such as improperly folded proteins. Plants have a range of chaperone proteins involved in the proper folding of other proteins (Park & Seo 2015). In the Caravel cultivar, we found the up-regulation of three molecular chaperones, namely 20 kDa chaperonin (CPN20, spot 20), chaperonin CPN60 (CPN60, spot 31), and heat shock cognate protein 70-kDa (HSC70, spot 35). On the other hand, in the Sary cultivar, we observed the down-regulation of heat shock 70 kDa protein 1 (HSP70, spot 35). Heat shock proteins (HSPs) are induced under stress conditions to prevent the accumulation of damaged proteins and to maintain cellular homoeostasis. Moreover, Ireland *et al.* (2004)

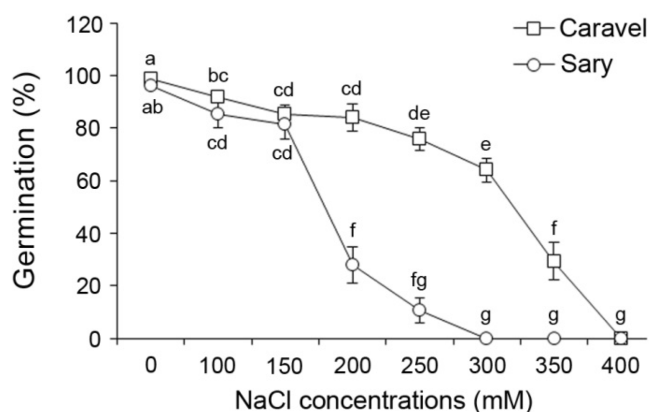

Fig. 1. Final germination percentages of the rapeseed cultivars at different NaCl concentrations (0–400 mM NaCl). Different letters (*a*–*g*) indicate significant differences according to DMRT.

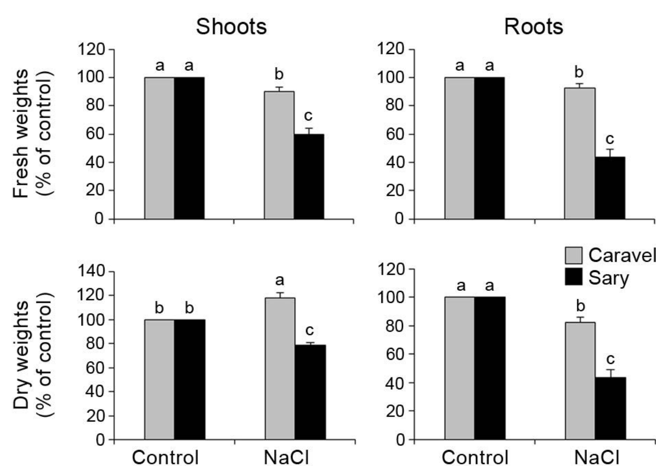

Fig. 2. Effects of NaCl stress (100 mM) on the fresh and dry weight of the shoots and roots of the rapeseed cultivars (Caravel and Sary) seedlings. Different letters indicate significant differences according to DMRT ($P < 0.05$)

suggested that HSP70 is a confirmed biomarker of NaCl stress. Chaperones play a vital role in repairing damaged proteins under stress conditions (Fernandez-Garcia *et al.* 2011). In contrast to our findings, a previous study identified CPN60 as an up-regulated protein in the roots of rapeseed under salinity (Bandehagh *et al.* 2013). Contrary to our findings, HSC70 protein was found to be down-regulated in the tolerant barley genotype, whereas it was up-regulated in the sensitive genotype (Mostek *et al.* 2015). In our study, the enhanced accumulation of chaperone-related proteins in the relatively tolerant cultivar may play a significant role in the adaptation of rapeseed seedlings to salinity stress at the germination stage. Our results also indicate that chaperones and HSPs could be used as potential markers of salt tolerance.

Some members of the proteasome system such as the 26S proteasome form part of the major proteolytic complex that degrades ubiquitin-tagged proteins (Collins & Goldberg 2017). In our study, proteasome subunit beta type-6 (spot 8), proteasome subunit alpha type-2-A-like (spot 13), proteasome subunit beta type-7-A-like (spot 14), and 26S protease regulatory subunit 7 homolog A-like (spot 26) were significantly up-regulated in only Sary, which indicates an increased rate of protein degradation in the sensitive cultivar. These proteins are needed for crucial cellular processes such as recycling redundant proteins and processing proteins from inactive to active states. In a previous study, several proteins related to proteolysis were seen to be up-regulated in salt stressed sugar beet suggesting that this proteasome pathway might help with degrading stress generated oxidized proteins (Yang *et al.* 2012). The enhanced abundance of proteasome-related proteins may indicate that salinity stress generated damaged proteins in the salt sensitive cultivar.

Plants can alleviate oxidative stress by activating stress defense mechanisms including antioxidant enzymes and other proteins. We found an important up-regulation of several antioxidant enzymes, superoxide dismutase [Fe] (FeSOD, spot 7) and L-ascorbate peroxidase 1 (APX, spot 15) under salt stress in the sensitive cultivar Sary, whereas the abundance of these proteins in the tolerant Caravel cultivar remained unchanged. Contradictory results were obtained by Mostek *et al.* (2015), who showed that most antioxidant enzymes exhibited decreased activity in a sensitive barley cultivar under salinity stress. Universal stress protein YxiE-like (spot 3), peroxiredoxin-2B-like (spot 4), NAD(P)H dehydrogenase (quinone) FQR1-like (spot 9) and glutathione S-transferase F3-like (GST, spot 11), which are involved in stress defense, were significantly increased under salinity stress only in the sensitive cultivar. Moreover, glutathione S-transferase U27-like (spot 12) was found to be up-regulated in Caravel, but down-regulated in Sary. GST enzymes play crucial roles in antioxidant signaling and stress tolerance in plants (Marrs 1996). Recently, it was shown that overexpression of GST genes increased drought and salinity tolerance in transgenic Arabidopsis (Xu *et al.* 2018). Here, our results indicate that the enhanced abundance of stress defense related proteins in the relatively sensitive cultivar may reflect the elevated oxidative burst under salinity stress.

Plants need a significant amount of energy to support their growth and development under stress conditions. Nevertheless, salinity stress results in the disruption of energy and carbohydrate metabolism in many plants (Zhang *et al.* 2019). In our study, we observed considerable diversity in the regulation of energy and carbohydrate metabolism including the tricarboxylic acid cycle, glycolysis, mitochondrial respiration, and the pentose phosphate pathway. Proteins such as mitochondrial ATP synthase subunit d (spot 5), malate dehydrogenase

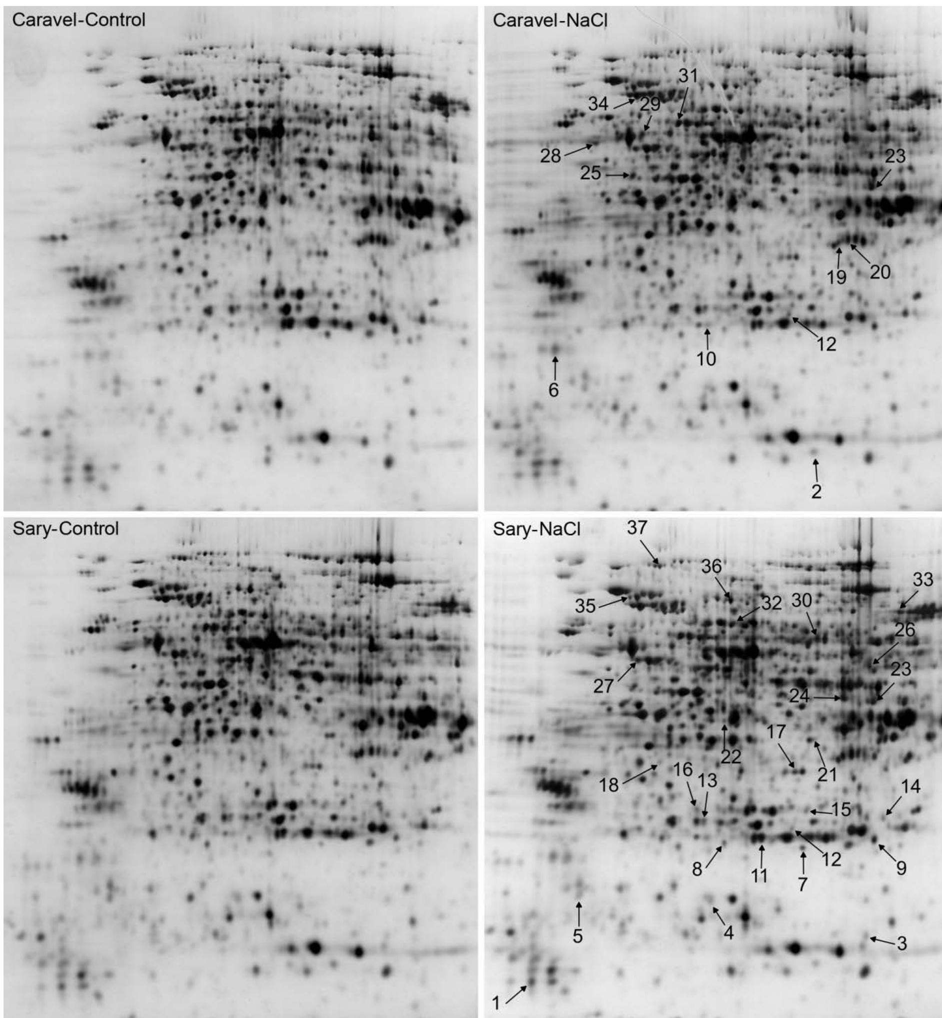

Fig. 3. Representative 2-D gel of differentially expressed protein in the rapeseed roots subjected to NaCl stress (100 mM). Total proteins were separated by 17 cm IPG strips (pH 4–7), followed by 12% SDS-PAGE and silver staining. Differentially expressed protein spots (1– 37) are marked by arrows

1 (MDH, spot 21), 2,3-bisphosphoglycerate-independent phosphoglycerate mutase 2 (spot 32), and transketolase-1 (spot 36) showed decreased abundance in the salt sensitive Sary cultivar, but remained unchanged in the salt tolerant cultivar during salt treatment. However, another isoform of malate dehydrogenase 1 (spot 20) was down-regulated only in the relatively tolerant Caravel cultivar under salinity stress. Mitochondrial ATP synthase is the key enzyme in energy metabolism, and enhanced accumulation of this enzyme provides the additional energy needed for cell homeostasis maintenance under stress conditions (Moghadam *et al.* 2012). Mostek *et al.* (2015) also found that ATP-synthase subunit accumulated less in a salt sensitive barley cultivar under salt stress. In addition to being an enzyme of the TCA cycle, MDH is also a key enzyme which controls the malate valve (Chen *et al.* 2015). Overall, the decreased levels of these proteins in Sary might be associated with the salinity-sensitive phenotype of this cultivar.

The modification of cell wall properties is one of the stress responses in plants. In the present study, an increased abundance of profilin-1-like (spot 1), tubulin alpha-6 chain (spot 27), and beta-glucosidase 23 (spot 33) were detected in Sary under salt stress, while an up-regulation of tubulin beta-4 chain-like (spot 28) was found only in Caravel. Profilin is an actin-binding protein and regulates cytoskeleton dynamics by polymerization or depolymerization of actin filaments (Ramachandran *et al.* 2000). Similarly, an increased abundance of profilin protein was reported in the sensitive genotype of barley (Fatehi *et al.* 2012). Alpha and beta chain tubulins are the major structural component of microtubules. A similar up-regulation pattern of tubulins was also shown in barley under salt stress (Mostek *et al.* 2015). These results suggest that the enhanced reorganization of the cytoskeleton structure may contribute to a plant's ability to withstand salinity stress.

Since salt stress can cause ammonium accumulation in roots (Debouba *et al.* 2007), ammonium assimilation is crucial for plant growth in terms of reducing toxicity (Bernard & Habash 2009). In our study, glutamate dehydrogenase 1 (GDH, spot 23) was significantly up-reg-

ulated in both of the rapeseed cultivars, while glutamate dehydrogenase 2-like (spot 24) was up-regulated only in Sary. GDH serves as the link between nitrogen and carbon metabolism by the amination of 2-oxoglutarate into glutamate or the deamination of glutamate into 2-oxoglutarate and ammonium (Debouba *et al*. 2007). Glutamate is also a precursor of proline, and the up-regulation of GDH may contribute to proline synthesis under salinity stress (Wang *et al*. 2007). Our results suggest that the up-regulation GDH proteins may enhance ammonium assimilation and the formation of osmoprotectants or supplementation of 2-oxoglutarate to the tricarboxylic acid cycle in rapeseed roots.

CONCLUSION

Two rapeseed cultivars, Caravel and Sary, were used as plant materials. Caravel showed a better germination rate and growth status under salt stress. A comparative proteomics analysis was performed on the roots of the rapeseed seedlings, and 37 differentially expressed proteins were identified. The majority of these proteins were involved in protein metabolism and stress response such as antioxidant enzymes. The relatively salt sensitive cultivar Sary displayed a high accumulation of proteins involved in antioxidant defense and the protein catabolic process such as FeSOD, APX, and different components of the proteasome system. On the other hand, the levels of molecular chaperones including CPN20, CPN60, HSC70, and HSP70 were higher in Caravel than Sary under salt stress. The rapeseed cultivars showed contrasting responses from the protective and regulatory mechanisms and the tolerant cultivar showed alterations to various protein groups leading to a more efficient system to cope with NaCl stress. Further studies to investigate the biological significance of these key proteins may contribute to a clearer understanding of the complex networks involved in germination under salinity stress.

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REZIME

Promene u proteomu korena kultivara *Brassica napus* pod stresom soli

Hakan Terzi i Mustafa Yildiz

Zaslanjivanje tla je važan ekološki problem koji utiče na poljoprivrednu proizvodnju širom sveta. Klijanje semena je kritičan proces, a uspostavljanje sadnica u slanim uslovima može se postići uspešnim klijanjem. U ovoj studiji, uporedna proteomika u kombinaciji sa fiziološkim analizama korišćena je za ispitivanje promena proteina u klijajućim sortama *B. napus* (Caravel i Sari) pod stresom NaCl. Klijanje semena se smanjuje sa povećanjem koncentracije NaCl. Međutim, Caravel je pokazao bolje performanse u pogledu klijavosti semena i rasta klijanaca pod stresom soli. Otuda je Caravel tolerantiniji na salinitet od sorte Sary. Proteini korena su ekstrahovani iz sorti *B. napus* koji su klijali na medijumu za rast biljaka sa ili bez 100 mM NaCl tokom sedam dana. Nakon što su protein korena izolovani pomoću dvodimenzionalne (2-D) gel elektroforeze, diferencijalno akumulirani proteini identifikovani su pomoću MALDI-TOF / TOF MS. Komparativnom analizom proteomike, 12 i 27 proteina bilo je statistički značajno akumulirano u NaCl tretiranim korenima Caravela i Sari-a. Identifikovani protein uglavnom učestvuju u metabolizmu proteina, odbrani od stresa, citoskeletu i ćelijskom zidu, kao i u metabolizmu energije. Sary kultivari pokazuju povećanje akumuliranih proteina koji su uključeni u antioksidativnu odbranu i katabolički proces proteina, poput superoksid dismutaze [Fe], L-askorbat peroksidaze 1 i različitih komponenti proteasomskog sistema. S druge strane, nivoi molekularnih čaperona, uključujući 20 kDa čaperon, čaperon CPN60, srodni protein toplotnog šoka HSC70 i toplotni šok 70 kDa protein 1, pod stresom soli su bili veći u Caravelu nego u kultivaru Sary. Ova otkrića će pružiti moguće mehanizme koji doprinose toleranciji na so i mogu poslužiti kao osnova za poboljšanje tolerancije na slanost uljane repice.

Ključne reči: *Brassica napus*, klijanje, proteomika, tolerancija soli