Analyses of Inter-cultivar Variation for Salinity Tolerance in Six Korean Rapeseed Cultivars

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Abstract. Salinity stress is one of the most serious factors limiting the productivity of agricultural crops. The aim of this study was to assess inter-cultivar (intraspecific) variation for salinity tolerance in six Korean rapeseed (Brassica napus L.) cultivars at the seedling stage. The effect of three different salinity stress levels (EC 4, 8, and 16 dS·m⁻¹) on seedlings of six cultivars was investigated through leaf size, leaf dry weight, and leaf chlorosis. At the highest salinity level (16 dS·m⁻¹), the mean decrease of leaf dry weight in ‘Sunmang’, ‘Tammi’, ‘Tamla’, ‘Naehan’, ‘Youngsan’, and ‘Halla’ was about 56.2, 56.9, 78.4, 79.3, 77.4, and 80.9%, respectively. ‘Tammi’ and ‘Sunmang’ showed much less reduction in leaf dry weight than all the other cultivars. In addition, diluted seawater treatments increased the occurrence of leaf chlorosis in six cultivars. At EC 8 and 16 dS·m⁻¹, ‘Naehan’, ‘Youngsan’, and ‘Halla’ showed a higher level of leaf chlorosis than ‘Tammi’, ‘Sunmang’, and ‘Tamla’. On the basis of these results, six cultivars were placed into salinity-tolerant and sensitive groups. ‘Tammi’ and ‘Sunmang’ were the salinity-tolerant cultivars, while ‘Naehan’, ‘Halla’, ‘Youngsan’, and ‘Tamla’ were the salinity-sensitive cultivars. ‘Tammi’ and ‘Naehan’ rated as the most tolerant and most sensitive cultivar, respectively. To further analyze protein expression profiles in ‘Tammi’ and ‘Naehan’, 2-D proteomic analysis was performed using the plants grown under diluted seawater treatments. We identified eight differentially displayed proteins that participate in photosynthesis, carbon assimilation, starch and sucrose metabolism, amino acid metabolism, cold and oxidative stress, and calcium signaling. The differential protein expressions in ‘Tammi’ and ‘Naehan’ are likely to correlate with the differential growth responses of both cultivars to salinity stress. These data suggest that ‘Tammi’ is better adapted to salinity stressed environments than ‘Naehan’.

Additional key words: Brassica, seawater

Introduction

Soil salinity is one of the most severe environmental stress factors limiting the productivity of agricultural crops. The harmful effects of salt on plants are a consequence of both a water deficiency resulting in osmotic stress and the effects of excessive accumulation of sodium ions on critical biochemical processes (Apse et al., 1999; Ashraf and McNeilly, 2004). A number of studies have shown that salt-tolerant plants are able to prevent excessive accumulation of Na⁺ in cytosol, thus sustain the ion homeostasis in plant cells. AtNHX1, a vacuolar Na⁺/H⁺ antiporter gene of Arabidopsis, was firstly isolated (Gaxiola et al., 1999). Overexpression of AtNHX1 improved salt tolerance in Arabidopsis (Apse et al., 1999), tomato (Zhang and Blumwald, 2001), rapeseed (Zhang et al., 2001), and petunia (Xu et al., 2009). In response to high salinity stress, various genes become expressed, the products of which are involved in plant protection (Tuteja, 2007). Some of the genes encoding osmolytes, ion channels, receptors, components of calcium signaling, and some other regulatory signaling factors or enzymes are able to confer salinity-tolerant phenotypes when transferred to sensitive plants (Tuteja, 2007).

Changes in protein expression profiles can declare, to a
great extent, the responsible bands for resistance to salinity. Abdel-Hady (2001) found that salt treatment induced clear modifications in the protein pattern of wheat callus. Albassam (2001) reported that salinity reduced the activity of nitrite reductase (NiR) in pearl millet which was irrigated with nutrient solution containing 0, 25, 50, or 100 mM NaCl. Khan and Gulzar (2003) found that, presence of NaCl around roots leads to degradation of some protein involved in root and shoot growth.

Salinity has adverse effects on germination and emergence among all the potential oilseed Brassica species. Most of the Brassica species have been categorized as moderately salt tolerant, with the amphidiploid species being the relatively salt tolerant in comparison with the diploid species (Ashraf and McNeill, 2004). Their salt tolerance has been acquired from the A (B. campestris) and C (B. oleracea L.) genomes (Ashraf and McNeill, 2004). Six Brassica species, B. napus, B. campestris, B. nigra, B. juncea, B. oleracea, and B. carinata, were examined by He and Cramer (1992), and they found that B. napus and B. carinata were the most tolerant and most sensitive species, respectively. Intraspecific and interspecific variation for salt tolerance is present to a great extent in these crops (Ashraf and McNeill, 2004; Bybordi and Tabatabaei, 2009). Little information was available on the tolerance of Korean rapeseed cultivars to salinity. Therefore, the present study was conducted to evaluate the effect of salinity on seedling growth of six Korean rapeseed cultivars under high salinity conditions and to investigate their inter-cultivar variation for salinity tolerance and relative responses to salinity at the proteomic level.

Materials and Methods

Seawater Irrigation of Six Korean B. napus Cultivars

Six Korean B. napus cultivars, ‘Sunnang’, ‘Tammi’, ‘Tamla’, ‘Naehan’, ‘Youngsan’, and ‘Halla’, were used to assess their salinity stress tolerance. The experiment was conducted at the Bioenergy Crop Research Center of the National Institute of Crop Science in 2009/2010. The seeds were directly sown in the greenhouse at the end of October as shown in Fig. 1 and allowed to grow for four weeks under groundwater condition. And then 30 L of diluted seawater (EC 4, 8, and 16 dS·m⁻¹) was irrigated once a week for eight weeks. Control plants were irrigated only with ground water. Plants were applied by drip irrigation system.

Measurements of Factors

Leaf length (with petiole) and width were measured after eight weeks of diluted seawater treatments (about 90 days after sowing). The number of leaves with visual symptoms such as chlorosis was counted. For measuring the leaf dry weight, leaves were placed in a 70°C oven for 48 h and the dry weight of leaves was measured to the nearest 0.1 g.

Protein Sample Preparation

For analyzing the protein, leaves were placed in a -20°C freezer for 12 h and lyophilized in a -75°C freeze dryer for 48 h (IIShinBioBase Co., Ltd., Korea). The plant tissues were homogenized directly by mortar-driven homogenizer (PowerGen125, Fisher Scientific). Sample analysis solution was composed of 7 M urea, 2 M thiourea containing 4% (w/v) 3-[(3-cholamidopropy) dimethyammonio]-1-propanesulfonate (CHAPS), 1% (w/v) dithiothreitol (DTT), and 1% pharmalyte and 1 mM benzamidine. Proteins were extracted for 1 h at room temperature by vortexing. After centrifugation at 15,000 g for 1 h at 15°C, insoluble material was discarded and the soluble fraction was used for two-dimensional gel electrophoresis. Protein concentration was normalized by Bradford assay (Bradford, 1976).

2D (Two–dimensional Gel Electrophoresis) Page

IPG dry strips were equilibrated for 12-16 h with 7 M urea, 2 M thiourea containing 2% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 1% dithiothreitol (DTT), 1% pharmalyte and loaded with 200 µg of sample respectively. Isoelectric focusing (IEF) was performed at 20°C using a Multiphor II electrophoresis unit and EPS 3500 XL power supply (Amersham Biosciences) following manufacturer’s instruction. For IEF, the voltage was linearly increased.
from 150 to 3,500 V for 3 h for the sample entry followed by a constant 3,500 V, with focusing complete after 96 kVh. Prior to the second dimension (gel electrophoresis), strips were incubated for 10 min in equilibration buffer (50 mM Tris-Cl, pH 6.8 containing 6 M urea, 2% SDS and 30% glycerol), first with 1% DTT and second with 2.5% iodoacetamide. Equilibrated strips were inserted onto SDS-PAGE gels (20 × 24 cm, 10-16%). SDS-PAGE was performed using Hoefer DALT 2D system (Amersham Biosciences) following the manufacturer’s instruction. 2D gels were run at 20°C for 1,700 Vh. And then, 2D gels were coomassie G250 stained as described by Anderson et al. (1991).

Image Analysis

Quantitative analysis of digital images was carried out using the PDQuest (version 7.0, BioRad) software according to the protocols provided by the manufacturer. Quantity of each spot was normalized by total valid spot intensity. Protein spots were selected for their significant expression variation deviated over two fold in its expression level compared with the control sample.

Enzymatic Digestion of Proteins

Protein spots were enzymatically digested in-gel in a manner similar to that previously described by Shevchenko et al. (1996) and using modified porcine trypsin (Promega modified). Gel pieces were washed with 50% acetonitrile to remove SDS, salt and stain. Washed and dehydrated spots were then vacuum dried to remove solvent and rehydrated with trypsin (8-10 ng·µL⁻¹) solution in 50mM ammonium bicarbonate pH 8.7 and incubated for 8-10 h at 37°C.

Mass Spectrometry

Samples were analyzed using the Applied Biosystems 4700 proteomics analyzer with TOF/TOF™ ion optics. Both MS and MS/MS data were acquired with a Nd:YAG laser with 200 Hz repetition rate, and up to 4000 shots were accumulated for each spectrum. MS/MS mode was operated with 2 keV collision energy; air was used as the collision gas so that nominally single collision conditions were achieved. Although the precursor selection has a possible resolution of 200, in these studies of known single component analytes a resolution of 100 was utilized. Both MS and MS/MS data were acquired using the instrument default calibration, without applying internal or external calibration. Sequence tag searches were performed with the program MASCOT (http://www.matrixscience.com).

Quantitative Real-time Polymerase Chain Reaction (PCR)

Cu/Zn superoxide dismutase (Cu/ZnSOD) gene expression was quantified using the Thermal Cycler Dice Real Time System (Takara, Japan). Cu/ZnSOD gene expression was measured. Total RNA was extracted using the RNAeasy Plant Mini kit (Qiagen). After DNase I (Takara, Japan) treatment, cDNA was synthesized using the Invitrogen superscript double-stranded cDNA synthesis kit. Quantitative real-time PCR was carried out by the Thermal Cycler Dice Real Time System (Takara, Japan). A pair of Cu/ZnSOD primers (BnSOD-F: cctggtccccatggtttccatgtc, BnSOD-R: agaacaagggcgcctggtttcc) were designed from B. napus Cu/ZnSOD (accession: AY970822) and were used for determination of expression levels of Cu/ZnSOD in the plants.

Results

Effects of High Salinity on Seedling Growth of Six Korean Rapeseed Cultivars

Increasing salinity levels of irrigation water progressively depressed the seedling growth of the six Korean rapeseed cultivars. The most common adverse effects of salinity on the six cultivars were the reduction in leaf size and dry matter accumulation. The appearance of the plants at eight weeks after diluted seawater treatments (EC 4, 8, and 16 dS·m⁻¹) is shown in Fig. 2. The average leaf lengths of all the cultivars at 4, 8 and 16 dS·m⁻¹ were about 22, 17, 13, and 8 cm, respectively (Fig. 3A) and the average of leaf widths were about 9, 7, 6, and 4 cm, respectively (Fig. 3B). As a result of the reduction in leaf growth, leaf dry weight of six cultivars declined rapidly with increasing salinity levels (Fig. 3C). Their leaf dry weights were significantly reduced at EC 8 dS·m⁻¹ and above. The decreasing rate was obviously different among cultivars. At the highest salinity level (16 dS·m⁻¹), the average of leaf dry weight in ‘Sunmang’, ‘Tammi’, ‘Tama’, ‘Naehan’, ‘Youngsan’, and ‘Halla’ is 1.35, 1.24, 0.68, 0.60, 0.65, and 0.58 g, respectively. The mean decrease of leaf dry weight in the six cultivars was about 56.2, 56.9, 78.4, 79.3, 77.4, and 80.9%, respectively. ‘Tammi’ and ‘Sunmang’ showed less reduction in leaf dry weight than ‘Naehan’, ‘Halla’, ‘Youngsan’, and ‘Tama’. In addition, leaf chlorosis, which is a yellowing of leaf tissue due to a lack of chlorophyll, was observed for all the cultivars at EC16 dS·m⁻¹ (Fig. 3D). Symptoms of leaf chlorosis were highly observed in ‘Naehan’, ‘Youngsan’, and ‘Halla’ at EC 8 dS·m⁻¹ and above, whereas it was shown in ‘Tammi’ at the lowest level (Fig. 3D). On the basis of relative growth responses to all the diluted seawater treatments used in this experiment, six cultivars could be roughly categorized into salinity tolerant and sensitive cultivars. ‘Tammi’ and ‘Sunmang’ were the salinity-tolerant cultivars, while ‘Naehan’, ‘Halla’, ‘Youngsan’, and
Fig. 2. Growth responses to salinity in six Korean *B. napus* cultivars, ‘Sunmang’ (A), ‘Tammi’ (B), ‘Tamla’ (C), ‘Naehan’ (D), ‘Youngsan’ (E), and ‘Halla’ (F). The plants were applied regularly with diluted seawater treatments (EC 4, 8, and 16 dS·m⁻¹) by drip irrigation system. Control plants were irrigated with groundwater. Bars = 30 cm in (A), (B), (C), (D), (E), and (F).

Fig. 3. The effect of salinity stress on the growth of six Korean rapeseed cultivars. Measurements of leaf length (A), leaf width (B), leaf dry weight (D), and occurrence of leaf chlorosis (D), were taken two months after diluted seawater treatments. Error bars represent the measurement range of six replicates.
‘Tamla’ were the salinity-sensitive cultivars. Overall, ‘Tammi’ and ‘Naehan’ were shown to be the most tolerant and sensitive cultivar in response to salinity stress, respectively.

Proteomic Pattern-based Analysis of Salinity Tolerance in ‘Tammi’ and ‘Naehan’ Cultivars

To further analyze the salinity responses between ‘Tammi’ and ‘Naehan’ at the proteomic level, we carried out 2-D proteomic analysis on both cultivars grown under diluted seawater treatments (Fig. 4). 2-D proteomic analysis was able to detect over approximately 168 protein spots. The comparison of the protein expression profiles of two cultivars revealed some changes in individual protein abundance and differences in protein expression profiles. Parts of the 2-D gel images are shown in Fig. 5, clearly showing changes and differences in protein intensities between ‘Tammi’ and ‘Naehan’ under diluted seawater treatments. The overall intensities of the eight protein spots were stronger in ‘Tammi’ than ‘Naehan’. At EC 16 dS·m⁻¹, protein staining intensities were much stronger in ‘Tammi’ than ‘Naehan’. These data suggested that the expression of eight proteins is relatively higher in ‘Tammi’ than ‘Naehan’ under high

![Image of 2-D gel analysis](image-url)

**Fig. 4.** Proteomic profiles of whole plant proteins from ‘Tammi’ (A) and ‘Naehan’ (B) cultivars grown under diluted seawater treatments for two months. Whole plant lysates were resolved on 2-D gels and silver stained.

![Image of 2-D gel analysis](image-url)

**Fig. 5.** Parts of the 2-D gel images showing changes and differences in protein intensities between ‘Tammi’ and ‘Naehan’. The expressions of eight protein spots are depicted with their spot numbers.
salinity stress.

To identify those spots, the protein spots on the reference gel were excised, digested in-gel with trypsin, and subjected to MALDI-TOF analysis (Fig. 6). The peptide profiles were used for database searches, and the list of 8 protein spots identified is summarized in Table 1. The listed proteins were glycine decarboxylase P-protein, aspartate aminotransferase, ferredoxin NADP⁺ reductase, sucrose-6F-phosphate phosphohydrolase, Cu/Zn-superoxide dismutase, actin depolymerizing factor, glycine-rich RNA-binding protein, and cold, circadian rhythm and RNA binding protein. The proteins had diverse functions, including carbon assimilation, starch and sucrose metabolism, photosynthesis, amino acid metabolism, cold and oxidative stress, calcium signaling and so on (Abarca et al., 2001; Engel et al., 2007; Goulas et al., 2006; Lunn et al., 2000; Uno et al., 2009). Furthermore, the differential protein expressions in ‘Tammi’ and ‘Naehan’ are likely to correlate with the differential response of ‘Tammi’ and ‘Naehan’ to salinity stress. These data are consistent with the results that ‘Naehan’ had suffered more salinity damage than ‘Tammi’.

Table 1. List of protein spots showing changes and differences in protein intensities between ‘Tammi’ and ‘Naehan’ cultivars.

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>Accession no.</th>
<th>Protein description</th>
<th>Sequence coverage (%)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>7820</td>
<td>AAU94363</td>
<td>glycine decarboxylase P-protein</td>
<td>8</td>
<td>51</td>
</tr>
<tr>
<td>7416</td>
<td>NP_194927</td>
<td>aspartate aminotransferase</td>
<td>24</td>
<td>262</td>
</tr>
<tr>
<td>6312</td>
<td>AAF79911</td>
<td>ferredoxin-NADP⁺ reductase</td>
<td>15</td>
<td>72</td>
</tr>
<tr>
<td>5509</td>
<td>AAG31075</td>
<td>sucrose-phosphatase</td>
<td>6</td>
<td>72</td>
</tr>
<tr>
<td>5003</td>
<td>ADR01108</td>
<td>Cu/ZnSOD (copper/zinc superoxide dismutase)</td>
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<td>117</td>
</tr>
<tr>
<td>4011</td>
<td>NP_851227</td>
<td>ADF3 (actin depolymerizing factor3)</td>
<td>8</td>
<td>49</td>
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<td>2016</td>
<td>P49311</td>
<td>GRP2A (glycine-rich RNA-binding protein)</td>
<td>7</td>
<td>44</td>
</tr>
<tr>
<td>201</td>
<td>NP_179760</td>
<td>CCR2 (cold, circadian rhythm and RNA binding 2)</td>
<td>22</td>
<td>124</td>
</tr>
</tbody>
</table>

Fig. 6. The 2-D reference map for ‘Tammi’ and ‘Naehan’ cultivars. A master gel was run with a mixture of equal aliquots from the whole plant lysates of the plants grown under different salinity treatments. Protein spots identified by MALDI-TOF MS analysis are indicated with numbers.

Fig. 7. Relative expression levels of Cu/ZnSOD transcripts in ‘Tammi’ and ‘Naehan’ cultivars. Expression levels were normalized to percent (100% is the level of Cu/ZnSOD transcripts in ground water-treated plants). Error bars represent the measurement range of two independent experiments.

Analysis of Cu/ZnSOD Expression Pattern between ‘Tammi’ and ‘Naehan’

To test the differential growth responses between ‘Tammi’ and ‘Naehan’ cultivars to salinity stress at a transcript level, the expression of Cu/ZnSOD mRNA was determined using quantitative real-time PCR (Fig. 7). cDNA was synthesized from a mixture of mRNA extracted from whole plants that are subjected to diluted seawater treatments (EC 4, 8, and 16 dS·m⁻¹). A primer pair (BnSOD-F:acctgtgcccatgtggtgc, BnSOD-R:agcaacacggcctctgcttc) was designed from B. napus Cu/ZnSOD (accession: AY970822) and was used for determination of relative expression patterns of Cu/ZnSOD transcripts in ‘Tammi’ and ‘Naehan’ cultivars. Different expression levels of Cu/ZnSOD were observed in ‘Tammi’ and ‘Naehan’. The expression level of Cu/ZnSOD in ‘Tammi’ increased at EC 4 and 8 dS·m⁻¹ and then decreased at EC 16 dS·m⁻¹, whereas the expression level in ‘Naehan’ increased at EC 4 dS·m⁻¹ and then decreased at EC 8 and
16 dS·m⁻¹. These results suggest that the expression pattern of Cu/ZnSOD under salinity stress were different between ‘Tammi’ and ‘Naehan’ cultivars.

**Discussion**

Most of the *Brassica* species have been categorized as moderately salt tolerant (Ashraf and McNeilly, 2004). However, little information is available on the tolerance of Korean rapeseed cultivars to salinity stress. Therefore, in the current study, six Korean rapeseed cultivars were evaluated to assess their intra-specific variation for salinity tolerance. Their salinity tolerance was examined by applying diluted seawater (EC 4, 8, and 16 dS·m⁻¹) and ground water as control (Fig. 1). Seawater treatments, particularly at high salinity levels (EC 8 and 16 dS·m⁻¹), significantly reduced leaf growth in the six cultivars (Figs. 2, 3A, and 3B). We found that decreasing rate of leaf dry weight is different among cultivars (Fig. 2C). The decreasing rate of leaf dry weight in ‘Tammi’ and ‘Sunmang’ was 56.2% and 56.9%, respectively, while the decreasing rate in ‘Tamla’, ‘Naehan’, ‘Youngsan’, and ‘Hall’ was 78.4, 79.3, 77.4, and 80.9%, respectively. These results suggested that, response of ‘Sunmang’ and ‘Tammi’ to salinity stress was more tolerant than ‘Naehan’, ‘Hall’, ‘Youngsan’ and ‘Tamla’. Bybordi and Tabatabaei (2009) investigated seedling responses of five rapeseed cultivars (Elite, Fornax, Licord, Okapi, and SLM046) to salinity stress levels (0 cont, 5, 10, 15 and 20 dS·m⁻¹). They found that increasing salinity decreased significantly radicle and plumule length and fresh weight. Decreasing rate was different among cultivars and tolerance ranking for cultivars was SLM046 > Okapi > Fornax > Licord > Elite. In addition, symptoms of leaf chlorosis were observed in the six cultivars under conditions of high salinity stress. The occurrence level of leaf chlorosis was totally different among six cultivars. Leaf chlorosis was highly observed in ‘Naehan’, ‘Youngsan’ and ‘Hall’ (Fig. 3D), whereas it was observed in ‘Tammi’ at the lowest level. These results indicate that ‘Tammi’ was more tolerant to salinity stress than ‘Naehan’. Some previous studies also indicated that one of the common adverse effects of salinity on trees and plants was the development of leaf chlorosis (Paludan-Müller et al., 2002; Vera-Estrella et al., 2005). Seedlings of four deciduous tree species maple (*Acer pseudoplatanus*), beech (*Fagus sylvatica*), horse chestnut (*Aesculus hippocastanum*) and lime (*Tilia cordata*) were exposed to de-icing salt (NaCl) either through the soil or applied to the above ground plant parts (Paludan-Müller et al., 2002). The development of leaf chlorosis covering up to 50% of the total leaf area was observed in salinity-sensitive species such as lime and beech. Vera-Estrella et al. (2005) also reported that after 2 weeks of salt treatment in *Thellungiella halophila*, leaf chlorosis was observed in plants treated with concentrations of NaCl above 200 mM.

To further analyze the salinity tolerance of ‘Tammi’ (cultivar showing high tolerance to salinity) and ‘Naehan’ (cultivar showing low tolerance to salinity) at the proteomic level, we employed 2D approach (Fig. 4). The proteomic pattern-based analysis showed that ‘Tammi’ has relatively higher protein expressions in glycine decarboxylase P-protein, aspartate aminotransferase, ferredoxin NADP⁺ reductase, sucrose-6F-phosphate phosphohydrolase, Cu/Zn-superoxide dismutase, actin depolymerizing factor, glycine-rich RNA-binding protein, and cold, circadian rhythm and RNA binding protein than ‘Naehan’ (Fig. 5). Glycine decarboxylase catalyzes the tetrahydrofolicate-dependent catabolism of glycine to 5, 10-methylene-tetrahydrofolute and the side products NADH, CO₂, and NH₃ (Engel et al., 2007). Aspartate aminotransferase (*At4g31990*) was increased in abundance during cold acclimation in Arabidopsis (Goulas et al., 2006). Ferredoxin NADP⁺ reductase is the last enzyme in the transfer of electrons during photosynthesis from photosystem I to NADPH. Sucrose-6F-phosphate phosphohydrolase catalyzes the final step in the pathway of sucrose biosynthesis and is the enzyme of photosynthetic carbon assimilation (Lunn et al., 2000). This reaction forms part of the photosynthetic cycle and contributes to one-carbon metabolism. Expression analysis of chloroplastic Cu/Zn-superoxide dismutase (SOD) gene in barley showed that it was induced in leaf tissues in response to drought, cold stress and exposure to stress related chemicals (Abu-Romman and Shatnawi, 2010). Superoxide dismutase is considered to be a crucial component in biological defense against oxidative stress. Actin depolymerizing factor3 (ADF3) in Arabidopsis interacts with calcium-dependent protein kinase that is essential sensor-transducers of calcium signaling pathways in plants (Uno et al., 2009). Overall, the proteins identified in our study had diverse functions including photosynthesis, carbon assimilation, starch and sucrose metabolism, amino acid metabolism, cold and oxidative stress, calcium signaling and so on (Abarca et al., 2001; Engel et al., 2007; Goulas et al., 2006; Lunn et al., 2000; Uno et al., 2009). Paludan-Müller et al. (2002) reported that salt treatment reduced photosynthesis up to 50% in salinity-sensitive species such as lime and beech. Proteins that are involved in photosynthesis and carbon assimilation were also found in our study.

The susceptibility or tolerance to high salinity stress in plants is a coordinated action of multiple stress responsive genes (Tuteja, 2007). Drought and salt stresses activate dehydration response element binding factor 2 (DREB2), members of the ethylene response factor (ERF)/APETALA2
(AP2) transcription factors family. DREB2 binds CRT/DRE promoter elements in stress response genes (Gosti et al., 1995; Yamaguchi-Shinozaki and Shinozaki, 2006). Salt stress, drought and, cold stress elevate abscisic acid (ABA) levels (Nambara and Marion-Poll, 2005). In some plant species, it has been observed that Cu/Zn-SOD expression is induced in response to a variety of chemical and environmental stimuli (Abu-Romman and Shatnawi, 2010). Whether the expression of proteins identified in this experiment was regulated by other environmental stimuli needs to be further analyzed. In this study, total 168 protein spots were detected by 2-D proteomic analysis. Further studies on classification of the protein spots into induced and deduced proteins under high salinity conditions will help to better understand effects of salinity on the proteomic level.

In conclusion, diluted seawater irrigation seriously affected seedling growth of six Korean rapeseed cultivars. ‘Tammi’ was the most tolerant cultivar with less response to the salinity applications on dry matter accumulation and leaf chlorosis, while ‘Naehan’ was the most sensitive cultivar. The 2-D proteomic analysis revealed that the overall intensities of the eight protein spots (sucrose-6F-phosphate phosphohydrolase, ferredoxin NADP+ reductase, glycine decarboxylase, aspartate aminotransferase, Cu/Zn-superoxide dismutase, actin depolymerizing factor, glycine-rich RNA-binding protein, and cold, circadian rhythm and RNA binding protein) were stronger in ‘Tammi’ than ‘Naehan’. These proteins are involved in photosynthesis, carbon assimilation, starch and sucrose metabolism, amino acid metabolism, cold and oxidative stress, calcium signaling and so on. The differential protein expressions in ‘Tammi’ and ‘Naehan’ are likely to correlate with the differential growth responses of ‘Tammi’ and ‘Naehan’ to salinity stress. The identified proteins in this study might have the potential to be used for improving crop’s salinity tolerance in areas where salinity is limiting factors for agricultural productivity.

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