Effects of NaCl on protein profiles of tetraploid and hexaploid wheat species and their diploid wild progenitors

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ABSTRACT

The soluble proteins extracted from the first leaf tissues of cultivated tetraploid (*Triticum durum* Desf., genome *AB*) and hexaploid (*T. aestivum* L., genome *ABD*) wheat species and their diploid wild progenitors [*T. monococcum* L. (*A*), *Aegilops speltoides* Tausch (*B*), and *Aegilops tauschii* Cosson (*D*)] exposed to 100 mmol/l NaCl stress were separated by two-dimensional (2D) gel electrophoresis. There was no newly synthesized protein in the NaCl treatment compared to the control treatment in all species. However, protein profiles showed some differences among species. Most of these proteins had acidic character; their isoelectric point (pI = pH – acidity of proteins) ranged between 5.1–6.9 and low-mol weight (LMW) between 20.3–30.6 kDa. Salt stress caused some proteins to increase or decrease. In the same MW and pI, 11 LMW and 3 intermediate-mol weight (IMW, 34.8–35.4 kDa) proteins increased and/or decreased in amounts were common between at least two species. The remarkable changes in *Ae. speltoides* were detected as decreases or losses in protein profiles. As a result of salt stress, all the remarkable changes in *T. durum* were detected as the increases in proteins. However, some proteins increased in *T. aestivum, T. monococcum* and *Ae. tauschii*. It is suggested that an increase in the amount of the proteins may lead to an increase in the tolerance mechanisms towards NaCl salinity of wheat species which has *A* and/or genome(s).

Keywords: salt stress; cultivated wheat species; wild wheat progenitors; electrophoresis; protein profile

Wheat (*Triticum* spp.) is one of the world's major cereal crops. Salinity is the key constraint to wheat production in irrigated agriculture in many parts of the world. Salt stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity. The tolerance to salt stress is accompanied by alterations in the levels of proteins. Salinity causes either decrease or increase in the level of soluble proteins or completely disappears in some proteins when compared to the control treatment (Yıldız 2007). In addition, salt stress promotes a complete loss of present proteins and the synthesis of newly formed proteins (Yıldız 2007). While some of genes whose expression is activated in response to salt stress encode for protective proteins such a osmotin (Zhu et al. 1995), late embryogenesis abundant (LEA) proteins (Zhu 2002) and ion transporters (Tester and Davenport 2003), others code for enzymes that participate in metabolic processes specifically triggered by saline stress (Gong et al. 2001). Accumulation levels of LEA proteins correlate with stress tolerance in various plant species suggesting protective roles under osmotic stress. Transgenic rice plants engineered to over-express barley *LEA* gene, *HVA1*, under control of the rice *actin 1* promoter exhibit better stress tolerance under 200 mmol/l NaCl and drought stress than wild-type plants (Xu et al. 1996).

Tetraploid wheat (*Triticum durum*, genome *AB*) was derived from a cross between diploid *T. monococcum* (*A*) and diploid *Aegilops speltoides* (*B*). Hexaploid wheat *T. aestivum* (*ABD*), on the other hand, clearly originates as a hybrid between *T. durum* (*AB*) and *Ae. tauschii* (*D*) (Gorham et al. 1991). In bread wheat, the *D* genome contains the *Kna1* locus on the long arm of chromosome 4D that confer higher salt tolerance to bread wheat by limiting the accumulation of Na^+ in the leaves (Dubcovsky et al. 1996).

The aim of this research was to present the changes in 2D electrophoretic profiles of the soluble proteins extracted from first leaf tissues

of tetraploid and hexaploid cultivated wheat species and their diploid wild progenitors grown in the presence or absence of NaCl.

MATERIAL AND METHODS

Plant materials and growth conditions

Seeds of cultivated tetraploid wheat species (*Triticum durum* Desf. cv. Aydın-93, genome *AB*) and hexaploid wheat species (*Triticum aestivum* L. cv. Gün-91, genome *ABD*) were obtained from Agricultural Research Institutes of Southeast Anatolia and Central Field Plants in Turkey, respectively. The ears of diploid wild progenitors of cultivated wheat [*Triticum monococcum* L. (*A*), *Aegilops speltoides* Tausch (*B*), and *Aegilops tauschii* Cosson (*D*); syn. *Ae. squarrosa*, *T. tauschii*)] were collected from Şanlıurfa (Southeast Anatolia Region) in Turkey.

Seeds were germinated at 25°C in dark on two filter papers in germination cups wetted with distilled water for four (*Ae. tauschii*) or two (other genotypes) days. After these periods, seedlings in the same physiological age were transferred to water culture medium. Control and salt treatment seedlings were placed into plastic cups (200 ml) including Hewitt solution without salt and with 100 mmol/l NaCl salt, respectively. All solutions were renewed every two days. Control and NaCl-treated seedlings were grown in a controlled growth chamber (25°C, 16 h photoperiod, 110 µmol photons/m^2/s), Sylvania Gro-Lux fluorescent lamp, F18W/GRO and 60% relative humidity) during 15 days. At the end of this period, the first leaves of control and NaCl-treated seedlings were sampled for electrophoresis analysis.

Protein extraction

The first leaf tissues (500 mg) of control and NaCl-treated seedlings of wheat genotypes were sampled, and total protein extraction was performed using the procedure of Damerval et al. (1986). In brief, leaf tissues were frozen and ground in liquid nitrogen. Total proteins were extracted by addition of 5 ml of 10% trichloroacetic acid in acetone containing 0.07% β-mercaptoethanol. After incubation at –20°C for 1 h, the suspension was centrifuged at 10 000 rpm (+4°C) for 15 min. Supernatant was discarded, and the pellet was resuspended with 5 ml acetone containing 0.07% β-mercaptoethanol, and kept at –20°C for 1 h. Centrifugation was repeated as above, and supernatant was decanted. Pellets were dried under vacuum, and kept at –20°C until use.

Protein estimation

Protein concentrations of extracts were determined by Ramagli and Rodriguez (1985). For protein determination, 5 mg of leaf tissue protein extracts were dissolved in 200 µl of urea mix solution (containing 9M urea, 1% ampholine pH 5–8, 1% ampholine pH 3–10, 4% Nonidet P-40, and 2% β-mercaptoethanol). The suspensions were incubated at room temperature for 15 min and then microcentrifuged at 12 000 rpm for 3 min. Supernatant aliquots (10 µl), 0.1N HCl (10 µl), distilled water (80 µl) and 1:3 diluted Bradford reagent (3.5 ml) were added to each sample tube. Blank tube was prepared without protein sample. Protein concentration of each sample was determined using Bovine Serum Albumin (BSA) standard curve. Optical density was recorded at 595 nm. Each sample was assayed with three replicates.

Two-dimensional electrophoresis

Total protein extracts were used for two-dimensional gel electrophoresis according to the method of Naqvi et al. (1994). The first dimension (IEF) was performed using rod gels (1.5 mm inner diameter and 180 mm length) at 400 V (3 h) followed by 800 V (15 h 30 min). Isoelectric focusing gel solution (IEFGS) consisted of 10 g urea, 3 ml of 30% acrylamide-bisacrylamide, 0.2 ml of ampholine (pH 5–8), 0.8 ml of ampholine (pH 3–10), 0.3 g of 3[3(cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 0.1 ml Nonidet P-40 (Sigma, St. Louis, MO) and 7.4 ml dH_2O (Hochstrasser et al. 1988). IEFGS was then filtered and degassed. Protein amounts were adjusted to approximately 50 µg soluble protein equivalent to BSA depending on the experiment, and loaded onto the gels. The sample gels were 400 µl IEFGS, 50 µl sample protein containing 50 µg soluble protein, 2.5 µl of 2.5% ammonium persulfate (APS) and 1.0 µl of N,N,N',N'-tetramethylethylenediamine (TEMED). To determine the pH gradient, blank gel without sample was prepared in the same manner. Cathodic solution was 0.02M NaOH and the anodic solution was 0.01M H_3PO_4. As for pH gradient, blank gel was sliced into 1 cm pieces, each piece

was transferred to 1.4 ml of 1M KCl solution, kept overnight at 4°C, and then the pH was measured at 25°C.

After isoelectric focusing, the second dimension (SDS-PAGE) was carried out on 12% acrylamide gels (160 × 160 × 1.5 mm) according to Laemmli (1970). Commercial mol weight markers (MW-SDS-70L) obtained from Sigma (St. Louis, MO) were prepared between 20.1–66 kDa and used to standard. Separation was performed at 10°C using 10 mA constant current per gel for the first 30 min and then 25 mA per gel for the rest of the run. For silver staining, the 2D gels were fixed and silver stained using the procedure of Blum et al. (1987). Relative mol weight (Mr = MW – molecular weight, kDa) and isoelectric point of differentially induced-proteins in NaCl-stressed seedlings were detected.

RESULTS AND DISCUSSION

The changes in polypeptide compositions of total leaf protein extracts of cultivated tetraploid (*T. durum*, genome *AB*) and hexaploid (*T. aestivum*, genome *ABD*) wheat species and their diploid wild progenitors [*T. monococcum* (*A*), *Ae. speltoides* (*B*) and *Ae. tauschii* (*D*)] exposed to 100 mmol/l NaCl stress were analyzed by high resolution 2D gel electrophoresis. At least three electrophoresis repeats were performed for each set of experiment. The gels that exhibited similar protein profiles were evaluated for the data.

Although the cultivated wheat species and their wild progenitors have different genomes, the protein profiles of these genotypes were similar. We demonstrated that there was no newly synthesized protein in the NaCl-treated seedlings compared to the control seedlings in all species. Similar results were reported in the leaves (Ouerghi et al. 2000) and roots (Majoul et al. 2000) of wheat when the seedlings were grown in 100 mmol/l NaCl for 21 days and in 200 mmol/l NaCl for 4 days, respectively. In contrast to our results, many newly synthesized proteins with low molecular weight were determined in the leaves of wheat (Yıldız 2007), the shoots and roots of barley (Ramagopal 1987) subjected to the NaCl treatments.

Some quantitative differences in 37 proteins were detected in the leaf protein profiles of the NaCl-treated seedlings compared to the control (Figure 1, Table 1). These proteins were quantitatively estimated and compared. As a result of salt stress, 21, 19, 11, 7 and 4 proteins showed quantitative differences in *T. monococcum*, *Ae. speltoides*, *T. durum*, *Ae. tauschii* and *T. aestivum*, respectively. Most of these proteins were of acidic character (pI 5.1–6.9) and low mol weight (LMW, 20.3–30.6 kDa). However, the changes in the amounts of five proteins with intermediate mol weight (IMW: 33.4–35.4 kDa, pI 6.6–7.8; denoted as proteins no. 33–37) were detected only in wild progenitors of wheat as a result of NaCl stress. Treatment of seedlings with NaCl resulted in an increase in the net synthesis of some proteins and a decrease in the synthesis of others. There were minor changes in the leaf protein profiles of *T. aestivum* (*ABD*) and *Ae. tauschii* (*D*) compared to other species. In *T. aestivum*, two proteins (20.7 kDa, pI 6.8 and 23.2 kDa, pI 6.5) were decreased, while two proteins (22.0 kDa, pI 6.3 and 23.2 kDa, pI 7.1) were increased. However, in *Ae. tauschii*, six proteins (20.7–35.3 kDa, pI 6.2–7.8) were increased, while one protein (23.2 kDa, pI 7.1) was decreased. Major changes in the protein profiles were determined in *T. monococcum*; nine proteins (23.2–35.4 kDa; pI 6.2–7.8) were increased, while 12 proteins (20.3–24.5 kDa, pI 5.1–6.6) were decreased. In *T. durum* (genome *AB*), all the changed proteins (11 proteins: 20.7–23.5 kDa, pI 5.1–7.1) were quantitatively increased as a result of NaCl salinity. In *Ae. speltoides* (genome *B*), 15 proteins (12 LMW: 20.7–30.3 kDa, pI 6.2–7.8 and 3 IMW: 34.8–35.4 kDa, pI 7.5–7.8) were quantitatively decreased, while four normal cellular proteins [LMW: 20.3, 20.7 and 21.0 kDa (proteins no: 1, 2 and 4, respectively) in pI 5.1 and 21.7 kDa (protein no. 9) in pI 6.9] were completely lost as a result of the NaCl treatment. Some researchers reported that the soluble protein contents with LMW, IMW and high molecular weight in leaves of different plant species decreased (Parida et al. 2002) or increased (Elshintinawy and Elshourbagy 2001) in response to salinity. However, the changes in protein synthesis were determined as new synthesis, complete loss, increase and decrease (Yıldız 2007). The most striking result in this research, the increases in amounts of some proteins, were determined in the leaf tissue profiles of *T. aestivum* (*ABD*), *T. durum* (*AB*), *T. monococcum* (*A*) and *Ae. tauschii* (*D*). It is suggested that the increases in the amount of proteins may lead to an increase in the tolerance mechanisms towards NaCl salinity of wheat species which has *A* and/or genome(s). Other important findings were following: (a) all of the proteins changed in the leaf protein profiles of *T. durum* (*AB*) were increased; (b) the number of the decreased proteins in *T. monococcum* (*A*)

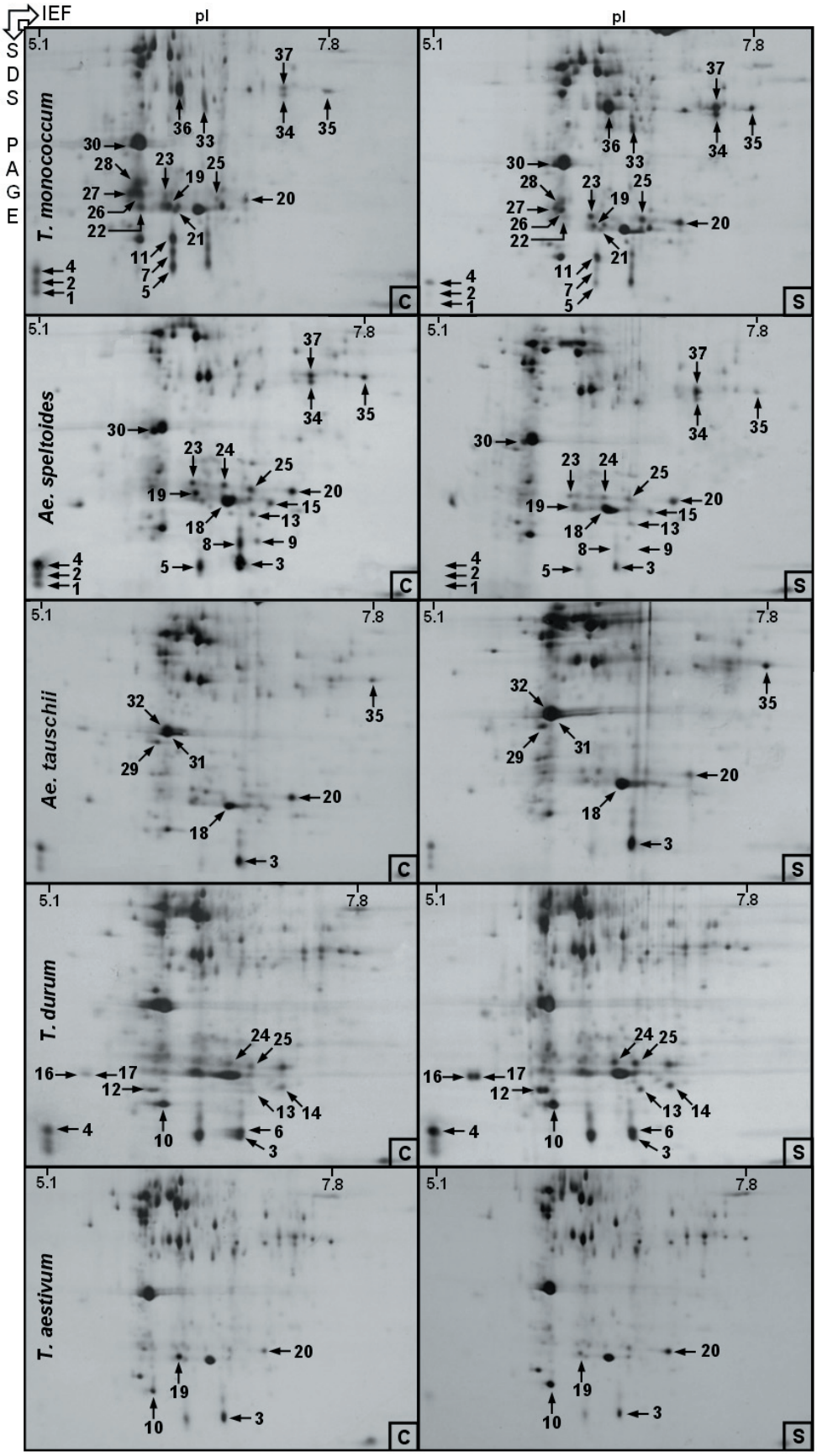

Figure 1. 2D profiles of the soluble leaf proteins extracted from control (C) and salt (S)-treated seedlings of *Triticum monococcum*, *Aegilops speltoides*, *Aegilops tauschii*, *Triticum durum* and *Triticum aestivum*. Increased, decreased and completely lost proteins in S compared to C treatment are indicated by arrow in both control and salt treatments (Table 1)

Table 1. Protein polymorphism in the leaf tissues of cultivated hexaploid and tetraploid wheat species and their diploid wild progenitors in 100 mmol/l NaCl treatment compared to the control treatment

Protein number	Mr	pI	Cultivated wheat species		Wild wheat species (progenitors)		
			Triticum aestivum (*AABBDD*)	*Triticum durum* (*AABB*)	*Triticum monococcum* (*AA*)	*Aegilops speltoides* (*BB*)	*Aegilops tauschii* (*DD*)
1	20.3	5.1			–*	CL*	
2	20.7	5.1			–	CL	
3	**20.7**	**6.8****	–	+*		–	+
4	**21.0**	**5.1**		+	–	CL	
5	**21.0**	**6.6**			–	–	
6	21.0	6.8		+			
7	21.5	6.6			–		
8	21.7	6.7				–	
9	21.7	6.9				CL	
10	**22.0**	**6.3**	+	+			
11	22.0	6.6			–		
12	22.5	6.2		+			
13	**22.5**	**6.8**		+		–	
14	22.7	7.1		+			
15	22.9	7.0				–	
16	23.0	5.4		+			
17	23.0	5.5		+			
18	**23.0**	**6.7**				–	+
19	**23.2**	**6.5**	–		–	–	
20	**23.2**	**7.1**	+		+	–	–
21	23.3	6.6			–		
22	23.5	6.3			–		
23	**23.5**	**6.5**			+	–	
24	23.5	6.7		+		–	
25	**23.5**	**6.8**		+	+	–	
26	23.8	6.3			–		
27	23.9	6.2			–		
28	24.5	6.3			–		
29	29.5	6.2					+
30	**30.3**	**6.2**			+	–	
31	30.4	6.3					+
32	30.6	6.3					+
33	33.4	6.7			+		
34	**34.8**	**7.5**			+	–	
35	**35.3**	**7.8**			+	–	+
36	35.4	6.6			+		
37	**35.4**	**7.5**			+	–	

*CL = completely lost protein; – = protein decreased in amount; + = protein increased in amount

**bold numbers in Mr and pI column indicate common proteins between at least two species

was higher than the increased proteins; and (c) the proteins changed in *Ae. speltoides* (*B*) were diminished or disappeared as a result of NaCl stress. In this sense, it is suggested that most of the decreased and disappeared LMW proteins may be salt sensitive in different degree. Also, this case may be explained with an increase in activities of protein hydrolyzing enzymes during the NaCl stress treatment. Parida et al. (2002) reported that the decrement in total soluble protein content of leaves as a result of the NaCl treatment might have resulted from an adverse effect of NaCl on the synthesis of certain LMW proteins. In addition, some researchers reported that the contents of two LMW proteins in hexaploid wheat, and 17 LMW and four IMW in tetraploid wheat decreased, while one LMW protein in hexaploid wheat and four LMW proteins in tetraploid wheat disappeared in the presence of NaCl (Elshintinawy and Elshourbagy 2001, Yıldız 2007).

In the same molecular weight and isoelectric point, 11 LMW (20.7–30.3 kDa, pI 5.1–7.1) and 3 IMW (34.8–35.4 kDa in basic character) proteins increased and/or decreased in amounts were common between at least two species in the NaCl treatment compared to the control treatment (Figure 1, Table 1). Other changes were detected as specific to species. The common proteins, which were quantitatively increased or decreased due to the NaCl treatment in species, may possible play a role in various functions of the tolerance mechanism. We suggest that the increases in the amount of protein(s) may be involved in a defense mechanism in species towards NaCl salinity, the decreases in the amount of the same protein(s) may not be enough to cope with NaCl salinity. In addition, the decrement and loss of the protein may lead to an increase in sensitivity of a genotype towards NaCl salinity.

Understanding the molecular basis of salt-stress signaling and tolerance mechanisms is essential for breeding and genetic engineering of salt tolerance in crop plants. To understand the molecular basis for mechanisms of salt tolerance in plants, it is important to distinguish constitutive and induced differences among cultivated species/cultivars and their wild progenitors. The modifications in protein profiles as a result of NaCl salinity may enable to elucidate the mechanism of salt tolerance of genotypes. Protein expression varies depending on particular species, variety, growth stage, organ and cell organelle in particular environments. The expression profile is closely related to the function of proteins (Hirano et al. 2004). In this sense, one of the molecular approaches is an analysis of the plant proteome. Three major steps in the proteome analysis (developmental and environmental proteomics) are the separation of complex protein mixture by two-dimensional (2D) protein gel electrophoresis, characterization of the separated proteins by mass spectrometer (MS) and database searching. The initial approach (two-dimensional protein gel electrophoresis) at the proteomic level is still a valuable tool for further determination of genes potentially involved in salt tolerance.

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