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Responses of two barley cultivars differing in their salt tolerance to moderate and high salinities and subsequent recovery

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Abstract

Two barley (*Hordeum vulgare* L.) cultivars, Czech spring cv. Amulet and Syrian landrace Tadmor, were subject to different salinity treatments: *1*) the NaCl concentration was gradually increased from 0 (the control) to either 100 (a moderate salt stress) or 300 mM NaCl (a high salt stress), *2*) the NaCl concentration was increased directly either from 0 to 300 mM NaCl or from 100 to 300 mM NaCl, and *3*) a recovery when all variants were transferred back to control conditions and cultivated for seven additional days before sampling. The following parameters were determined: water saturation deficit (WSD), osmotic potential (ψ_s), leaf proline content, maximum quantum yield of photosystem (PS) II photochemistry (measured as variable to maximum chlorophyll *a* fluorescence ratio, F_v/F_m), and relative accumulation of dehydrins (DHN). Both quantitative and qualitative differences in dehydrins were found between NaCl-treated Amulet and Tadmor. A principal component analysis (PCA) of all experiment data revealed a differential ability of Amulet and Tadmor to recover after the 300 mM NaCl treatments indicating better salt tolerance in Tadmor. Correlation analyses have shown statistically significant correlations between WSD, ψ_s, proline, and DHN.

Additional key words: chlorophyll fluorescence, dehydrins, *Hordeum vulgare*, proline, osmotic potential, principal component analysis.

Introduction

Salinity represents an important agricultural problem affecting more than 7 % of agricultural land and nearly a one third of irrigated land worldwide (Munns 2002, Colmer *et al.* 2006). The adverse effects of salinity on plants lie in a decreased osmotic potential of ambient soil water which restricts plant water uptake by roots (osmotic effect) as well as in an increased content of ions, specifically Na^+, which penetrate the plasma membrane and accumulate inside the cell (ionic effect). Some plants respond to osmotic stress by osmotic adjustment (an accumulation of ions in the vacuole or compatible osmolytes in the cytoplasm). To cope with the ionic effect, ion exclusion or intracellular compartmentalization mechanisms are activated (reviewed in Munns 2002, 2005).

Of the major crops, barley generally exhibits a relatively high salt tolerance with respect to other cereal crops, such as wheat, rice, and maize. A NaCl concentration of 250 mM has been regarded as the limit for sustainable growth and development in barley (Munns 2002, Flowers 2004). There are significant differences between barley cultivars in acquired salinity tolerance as well as in their abilities to recover after a salt treatment (Colmer *et al.* 2006). Landraces originating from arid and semi-arid regions, *e.g.*, the Middle East, generally exhibit a higher salinity tolerance and a better ability to recover after a salt stress than European elite cultivars.

Salinity profoundly affects plant water status which

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Abbreviations: DHN - dehydrins; F_v/F_m - variable to maximum chlorophyll *a* fluorescence ratio; PCA - principal component analysis; PS - photosystem; WSD - water saturation deficit; ψ_s - osmotic potential.
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can be easily determined as water saturation deficit (WSD), and determination of osmotic potential (ψ_s) can characterize the osmotic adjustment. In C_3 plants, such as barley, salt-induced stomatal closure and an associated lack of available CO_2 lead to an inhibition of net photosynthetic rate P_N. A simple method of assessment of the efficiency of primary photosynthetic reactions lies in a measurement of chlorophyll *a* fluorescence, and a decrease of variable to maximum chlorophyll *a* fluorescence ratio (F_v/F_m) has been considered as marker of stress (Rizza *et al.* 2001). Salinity also leads to profound changes in signalling, gene expression, as well as proteome and metabolome composition (Zhu 2002, Yamaguchi-Shinozaki and Shinozaki 2006). Profound changes in the abundance of several photosynthesis-related proteins leading to a downregulation of photosynthesis have been found in proteomic studies in several crops subjected to salinity (Kim *et al.* 2005, Caruso *et al.* 2008). At protein level, dehydrin proteins (DHN) from the LEA-2 protein family were selected for salinity tolerance assessment since a relationship between dehydrin protein accumulation and salinity tolerance has been found in several plants (reviewed in Kosová *et al.* 2010). A relationship between a relative accumulation of Y_nSK_m dehydrin DHN5 as well as DHN5 phosphorylation and salinity tolerance was reported by Brini *et al.* (2007) in durum wheat. At metabolite level, proline accumulation in leaf tissues was selected for salinity tolerance assessment since proline is an important non-toxic N-containing low-molecular mass osmo-protectant accumulating to high amounts in barley during osmotic adjustment (Voetberg and Stewart 1984, Munns 2005).

Several complex transcriptomic and proteomic studies have been published on barley response to salinity (Ozturk *et al.* 2002, Walia *et al.* 2009, Rasoulnia *et al.* 2011) which have significantly contributed to the identification of key transcript and protein accumulation patterns under salt stress. However, a selection of only a few key parameters (WSD, ψ_s, F_v/F_m, and proline and dehydrin content) could be useful for the characterization of plant stress response as well as their ability to recover following the stress. The aim of the present study was an evaluation of salinity tolerance and a subsequent recovery of two barley cultivars of different genotypic background and geographical origin, Czech spring cv. Amulet and Syrian landrace Tadmor.

Material and methods

Barley (*Hordeum vulgare* L.) seeds of Amulet, a Czech spring cultivar, and Tadmor, a black-seeded cultivar derived from a Syrian landrace Arabi Aswad by the International Center for Research in the Dry Areas, were obtained from the breeding company *Selgen* (Stupice, Czech Republic) and from Dr. L. Holková, Mendel University (Brno, Czech Republic), respectively. The seeds were surface-sterilized with 1 % (m/v) sodium hypochlorite for 5 min followed by rinsing with distilled water. The seeds were let germinate on moist filter paper in the dark for 5 d under a temperature of 20 °C and then plants were grown hydroponically in continuously aerated pots in a growth chamber (*Tyler T-16/4*, Budapest, Hungary) under an irradiance of 350 μmol m^{-2} s^{-1}, a 12-h photoperiod, a temperature of 20 °C, and a relative humidity of 90 %. The pots were filled with a commercially available solution *Hydropon* (*Lovochemie*, Lovosice, Czech Republic) corresponding to the Hoagland 3 nutrient solution including microelements, the final dilution was 1:200 (v/v); pH of the solution was adjusted to 6.5 by addition of KOH as described previously (Prášil *et al.* 2005). The hydroponical solution was changed every third day in order to prevent nutrient depletion. All plants were first exposed to the control treatment (0 mM NaCl, ψ_s -0.047 MPa) for 7 d. NaCl was added to the hydroponical solution every second day in a gradually increasing manner by 50 mM NaCl up to the final concentrations of 100 mM NaCl (a moderate salt stress; ψ_s reached -0.447 MPa) and 300 mM NaCl (a high salt stress; ψ_s reached -1.47 MPa), respectively. Plants were sampled after 15 d (1st sampling). The alternative way were direct transfers from 0 to 300 mM NaCl and from 100 to 300 mM NaCl. All variants were sampled after 20 d (2nd sampling). A recovery treatment was conducted by transferring all the plants back to the control conditions for 7 d followed by 3rd sampling (27 d of the hydroponical cultivation) (for detail see Fig. 1 Suppl.).

All parameters studied were determined in the youngest fully-developed leaves (leaf segments without bases and tips). The accumulation of dehydrins was also determined in crown (a 1 cm long segment) and root (a 5 cm long segment from root tip) tissues.

Water saturation deficit (WSD) was determined in 1 cm leaf segments according to Slavík (1963) and calculated as the difference between the mass of fully water-saturated leaf segments (FM1) and the fresh mass of leaf segments sampled under the given experimental conditions (FM0) and divided by the difference between the FM1 and the dry mass (DM) of the same segments, *i.e.*, WSD [%] = [(FM1 - FM0) / (FM1 - DM)] × 100. Osmotic potential (ψ_s) was determined in frozen and thawed leaf segments using a *VAPRO* dew point osmometer (*Wescor*, Logan, Utah, USA).

Proline content was determined in frozen leaf samples by a modified approach described in Jiménez-Bremont *et al.* (2006). Briefly, 100 mg of fine-ground frozen leaf samples was added to 15 cm^3 of water in a glass tube and then heated at 90 °C for 45 min. Then the samples were centrifuged at 5 000 *g* for 10 min and to 5 cm^3 of the

supernatant, 10 cm^3 of a 70 mM fresh ninhydrin solution (ninhydrin dissolved in a mixture of 2.08 M ortho-phosphoric acid and 13.9 M acetic acid) was added and the solution was heated at 90 °C for 60 min. After subsequent cooling, 10 cm^3 of toluene was added to the solution and the tubes with the mixture were shaken for 60 min. The mixture was stored at 5 °C overnight and the proline content was determined spectrophotometrically (*Nano Photometer*, *Implen*, München, Germany) at 520 nm in the upper toluene layer using L-proline (*Sigma-Aldrich*, St. Louis, MO, USA) as standard.

Chlorophyll *a* fluorescence parameters were determined in dark adapted leaves (at least 30 min of dark adaptation) in the growth chamber during a night phase using a portable fluorometer *Fluorpen FP 100* (*Photon Systems Instruments*, Drasov, Czech Republic). Parameter F_v/F_m was automatically determined from OJIP curve.

Relative accumulation of dehydrins was determined by a densitometric analysis of immunoblots prepared from frozen leaf, crown, and root samples according to Kosová *et al.* (2008). Briefly, samples were ground using a mortar and pestle in liquid nitrogen with an addition of a 100 mM Tris-HCl extraction buffer, pH 8.8, with a protease inhibitor (complete EDTA-free protease inhibitor cocktail tablets from *Roche*, Basel, Switzerland). Centrifugation at 14 000 *g* and 4 °C for 20 min and sample boiling for 15 min were carried out to enrich the dehydrin fraction. Proteins soluble upon boiling were precipitated by cold acetone containing 1 % (v/v) mercaptoethanol, and dry pellets were dissolved in a Laemmli buffer. Protein content was determined using a *RC DC* protein assay kit (*Bio-Rad*, Hercules, CA, USA). Samples were loaded on 12.5 % (m/v) SDS-PAGE gels and run at 10 mA (a stacking gel) and 25 mA (a resolving gel) according to the standard procedure (Laemmli 1970). The resolved proteins were transferred to nitrocellulose membranes, blocked in 4.5 % (m/v) non-fat dry milk in Tris-buffered saline (TBS), incubated with a primary anti-dehydrin antibody (*Enzo Life Sciences*, Farmingdale, NY, USA), resolved in 1.5 % non-fat dry milk in 0.05 % (v/v) *Tween-20* in TBS (a final dilution 1:1 000) and with an immuno-blot assay kit with goat anti-rabbit *IgG* alkaline phosphatase (*Bio-Rad*) according to the manufacturer's instructions. The membranes with visualized dehydrin bands were scanned using a densitometer *GS-800* (*Bio-Rad*) and the images were analysed using *Quantity One v. 4.6.2* (*Bio-Rad*).

Each data set containing at least three independent measurements of a given parameter at each sampling date was analysed by two-way *ANOVA* (two genotypes and five treatments) and by multiple comparison using the Duncan´s multiple range test (α = 0.05). All parameters were subjected to Z-score transformation and analysed by the principal component analysis (PCA). The individual parameters were also compared by the correlation analysis to determine a Pearson´s coefficient (r) of either positive or negative linear correlation. *ANOVA*, the correlation analysis, and PCA were carried out using *STATISTICA v. 10* (*StatSoft, Inc*, Tulsa, OK, USA).

Results

A significant increase in leaf WSD (from 10 to 20 %, Fig. 1*A-C*) and decrease in ψ_s (the lowest values approaching -6 MPa, Fig. 1*D-F*) were observed in both the barley genotypes exposed to both 100 and 300 mM NaCl gradually, and especially directly. After 7 d of the recovery treatment, increased WSD and decreased ψ_s persisted in all the variants exposed to 300 mM NaCl in Amulet revealing significantly higher WSD and lower ψ_s values than in Tadmor.

The leaf proline content (Fig. 1*G-I*) significantly increased under the salt stress except for the gradual increase of NaCl concentration to 100 mM. The proline content increased from *ca.* 10 in the control up to 300 μmol g^{-1}(d.m.) in the directly increased NaCl concentration to 300 mM. However, the increase was higher in Tadmor than in Amulet. After 7 d of recovery, the proline content remained high in Amulet, whereas it significantly decreased in the corresponding Tadmor samples.

The F_v/F_m ratio (Fig. 1*J-L*) revealed no significant differences between the genotypes and treatments at 1st sampling and it was around 0.8. However, the F_v/F_m ratio decreased significantly in the direct treatments (0 - 300, or 100 - 300 mM NaCl) with a significantly more profound decrease in Amulet than in Tadmor. After 7 d of recovery, the F_v/F_m ratio was restored in Tadmor, whereas it remained low in the corresponding Amulet samples.

The salinity led to an induction of several dehydrins in both the barley genotypes (Figs. 2 and 3) with Tadmor reaching generally a higher accumulation of dehydrins than Amulet. Both the genotypes accumulated dehydrins of a high relative molecular mass (Mr) of 82 kD (DHN5) and several low Mr dehydrins. Qualitative differences were found in the range of low Mr dehydrins between the cultivars: Amulet accumulated proteins of apparent Mrs of 29, 21, 19, and 18 kD and Tadmor of 26, 21, 19, and 18 kD. Quantitative differences were found among the barley organs with crowns revealing generally the highest dehydrin accumulation under the given treatment, followed by leaves and roots with barely detectable dehydrin bands (Fig. 3). The densitometric analysis of the individual protein bands revealed the largest abundance of a dehydrin corresponding to DHN5. An increased dehydrin accumulation in all 300 mM NaCl treatments was found in 1st and especially in 2nd sampling. After 7 d

of recovery, the increased dehydrin content persisted in the variants previously exposed to 300 mM NaCl with Tadmor exhibiting a higher dehydrin content than Amulet.

The two-way *ANOVA* analysis showed a significant effect of the salt treatments on all characteristics studied. However, the genotypes (Amulet, Tadmor) were only differentiated in ψ_s, proline, and F_v/F_m values at the 0.05 level (Table 1 Suppl.). The correlation analysis of the individual parameters in all the experiment variants has revealed significant linear correlations between DHN and proline, DHN and ψ_s, ψ_s and proline, DHN and WSD, WSD and proline, WSD and ψ_s, F_v/F_m and proline, and F_v/F_m and ψ_s (Table 2 Suppl.).

The principal component analysis (PCA; Fig. 2 Suppl.) revealed a similar dynamics between DHN and proline (both revealing an increase under the salinity) and a looser similarity between F_v/F_m and ψ_s (both revealing a

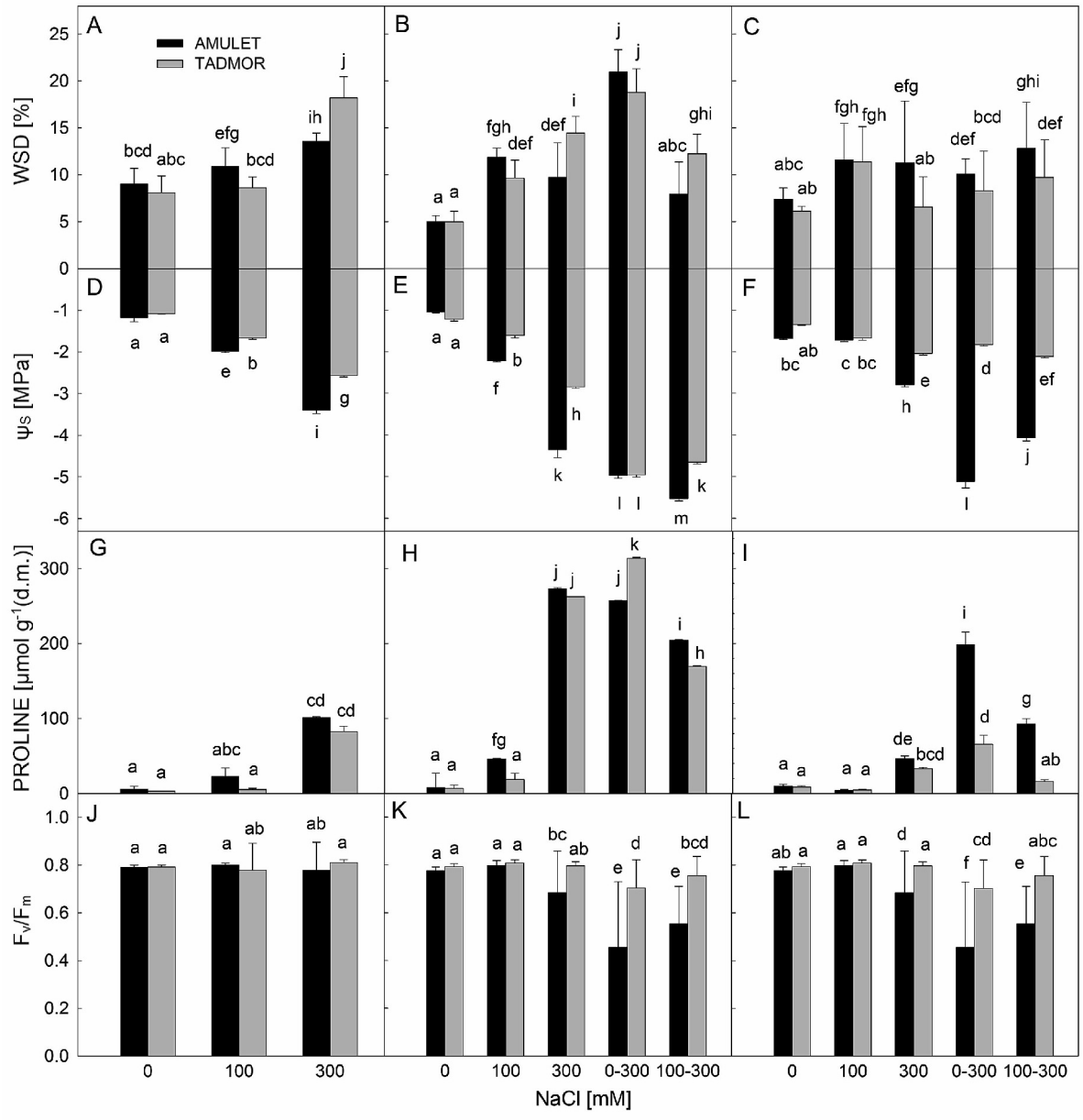

Fig. 1. Water saturation deficit (WSD; *A*,*B*,*C*), osmotic potential (ψ_s; *D*,*E*,*F*), proline accumulation (*G*,*H*,*I*) and maximum quantum yield of PS II photochemistry (F_v/F_m; *J*,*K*,*L*) at 1st sampling (*A*,*D*,*G*,*J*), 2nd sampling (*B*,*E*,*H*,*K*) and recovery (*C*,*F*,*I*,*L*) in Amulet (*black columns*) and Tadmor (*grey columns*) leaves. Treatments used were: 0 - control, 100 - a gradual transfer to 100 mM NaCl, 300 - a gradual transfer to 300 mM NaCl, 0-300 - a direct transfer from 0 to 300 mM NaCl, 100-300 - one-step transfers from 100 to 300 mM NaCl. Different letters indicate statistically significant differences within each parameter obtained by the Duncan's multiple range test ($P \leq 0.05$). *Error bars* indicate SE.

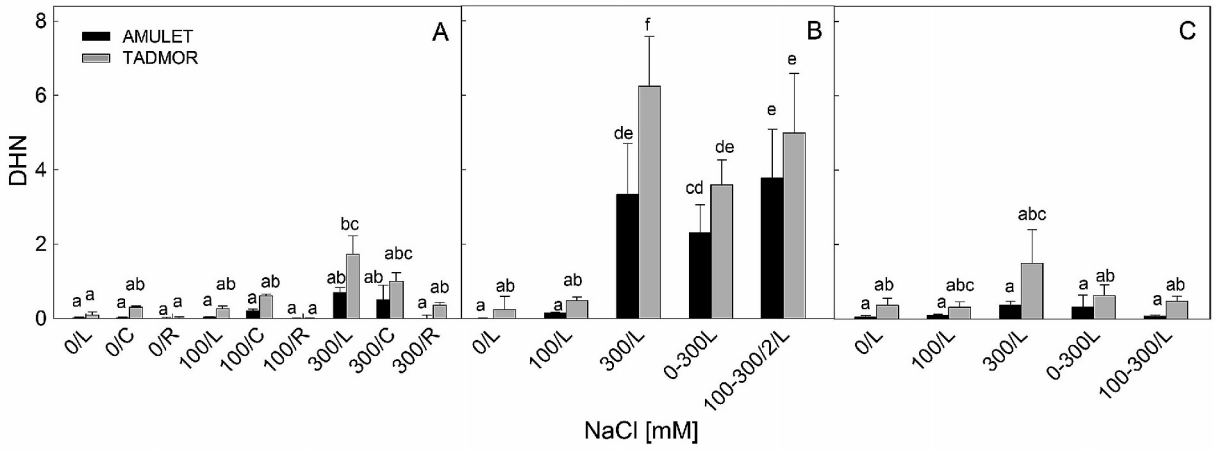

Fig. 2. Relative dehydrin accumulation at 1st sampling (*A*), 2nd sampling (*B*,) and 3rd sampling (*C*) in Amulet (*black columns*) and Tadmor (*grey columns*) leaves (L), crowns (C), and roots (R) at control (0), 100 mM NaCl (100), 300 mM NaCl (300), one-step transfers from 0 to 300 mM NaCl (0-300) and from 100 to 300 mM NaCl (100-300). Different letters indicate statistically significant differences obtained by the Duncan's multiple range test at $P \leq 0.05$. *Error bars* indicate SE. The relative accumulation of the sum of all DHN proteins was expressed to the relative accumulation of DHN proteins in Tadmor, crown, at 300 mM NaCl, and 1st sampling which was used as internal standard and whose content was set as 1.

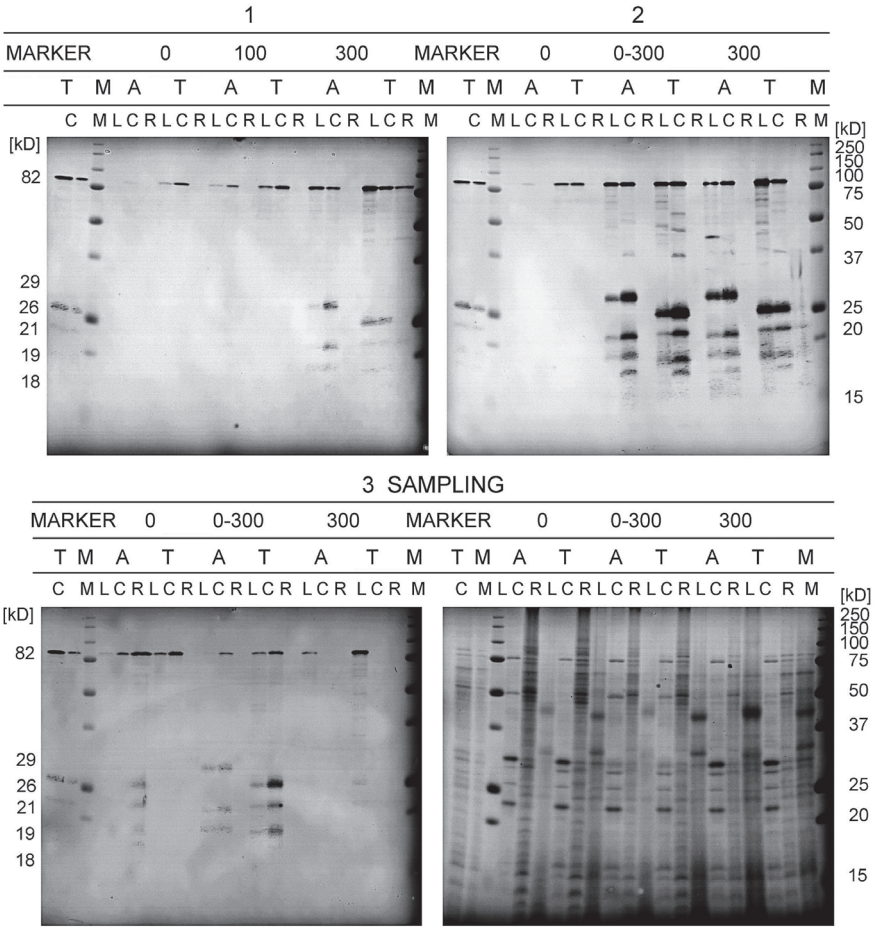

Figure 3. Representative immunoblots and a representative 1D SDS-PAGE gel showing a dehydrin protein accumulation in Amulet (A) and Tadmor (T) leaf (L), crown (C), and root (R) samples at different salinity treatments [0, 100, 300 mM NaCl; one-step transfer from 0 to 300 mM NaCl (0-300) at 1st sampling (1), 2nd sampling (2) and 3rd sampling (3)]. M means protein standard (all *Blue Precision Plus* protein standards; *Bio-Rad*). The sample Tadmor, crown, 300 mM NaCl, and 1st sampling was loaded on all gels as internal standard.

decrease under the salinity), whereas a differential dynamics in WSD with respect to the other parameters studied (Fig. 2 Suppl.). Two basic groups could be distinguished with one group encompassing control and recovered samples in the right half of the plot and the other group encompassing stressed plants in the left half of the plot. The ability of both Amulet and Tadmor to recover after the moderate salt (100 mM NaCl) stress is indicated by the position of 100/A/3 and 100/T/3 samples in the vicinity of the control samples in the right half of the PCA plot. Significant differences were found between the genotypes exposed to 300 mM NaCl and after the recovery. The Tadmor samples are placed in the vicinity of the control samples in the right half of the PCA plot, whereas the Amulet samples are placed in the vicinity of the stressed samples in the left half of the PCA plot.

Discussion

Salinity represents a severe osmotic and ionic stress on plants (Munns 2002, 2005, Munns and Tester 2008). The extent of plant tissue dehydration can be described by several parameters, such as relative water content (RWC) or WSD expressing the fraction of water needed for the full tissue hydration. The hypothesis on salt ion contribution to pressure potential maintenance is supported by relatively low WSD reaching the maximum of 20 % in the samples exposed to the high-salt stress. After recovery, a decrease in WSD in Tadmor indicated a rehydration of plant leaf tissue, whereas high WSD in Amulet exposed to 300 mM NaCl indicated an irreversible leaf tissue damage.

Plants try to restrict tissue dehydration by an accumulation of osmotically active compounds (Zhang *et al.* 1999). The opinions on a beneficial role of osmotic adjustment are contradictory (Serraj and Sinclair 2002, Lawlor 2013). Accumulation of some osmolytes is species-specific, *i.e.*, plants accumulate naturally only some kinds of osmolytes, whereas avoiding others (Munns 2005). Cereals accumulate glycinebetaine and proline under osmotic stress (Colmer *et al.* 2006, Islam *et al.* 2007). The accumulation of proline was highest at 300 mM NaCl (the 2nd sampling date) which corresponds to the lowest ψ_s (up to -6 MPa) measured in the same samples. Proline has been shown to participate on stabilization of antioxidant enzymes (Bose *et al.* 2014). A positive effect of proline accumulation on salt tolerance was reported by Kishor *et al.* (1995) and Hong *et al.* (2000) in tobacco transformants expressing a mutated form of proline biosynthetic enzyme pyrroline-5-carboxylate synthetase lacking a feedback inhibition. The transformants revealed an elevated proline content, a reduced accumulation of reactive oxygen species (ROS), as well as an ability to grow on a medium containing up to 200 mM NaCl. Similarly, Islam *et al.* (2007) found a higher proline content in salt-tolerant *Hordeum marinum* in comparison to *Triticum aestivum* plants grown at 200 mM NaCl. In contrast, De Lacerda *et al.* (2003) have concluded in the study on two sorghum genotypes subjected to 100 mM NaCl that proline accumulation appears to be a reaction on salt stress damage rather than associated with an enhanced salt tolerance. Chen *et al.* (2007) have found a higher proline content in salt-sensitive barley cultivars compared to salt-tolerant ones when plants were gradually acclimated to 320 mM NaCl. Our results show a higher proline content in Amulet compared to Tadmor which might indicate an association of proline accumulation with plant damage upon the salt stress. Persistence of increased proline content in the Amulet plants after the recovery treatment confirmed salt sensitivity of this cultivar.

Plants accumulate hydrophilic proteins from the LEA family under stress conditions associated with cell dehydration (Battaglia *et al.* 2008). Dehydrins from the LEAII family exhibit several protective functions in plant cells including chaperone, space-filling, cryoprotective, antifreeze, radical-scavenging, and ion-binding functions (Rorat 2006, Kosová *et al.* 2010). Dehydrin transcript accumulation under abiotic stresses was reported for nearly all of 13 dehydrin genes identified in barley except for embryo-specific *Dhn12* (Choi *et al.* 1999, Suprunova *et al.* 2004, Tommasini *et al.* 2008). The accumulation of dehydrins of high Mr of 82 kD corresponding to DHN5 was reported under frost, drought, salinity, and osmotic stresses (Van Zee *et al.* 1995, Bravo *et al.* 1999, Choi *et al.* 1999, Zhu *et al.* 2000, Kosová *et al.* 2008, 2010). Induction of low Mr dehydrins of the Y_nSK_m type (*Dhn1*, *Dhn2*, *Dhn4*, *Dhn6*, *Dhn7*, *Dhn9*, and *Dhn11*) was reported under dehydration stress at transcript level (Choi *et al.* 1999, Suprunova *et al.* 2004, Tommasini *et al.* 2008). At protein level, several low Mr mass dehydrins were detected but not identified (Bravo *et al.* 1999, Zhu *et al.* 2000, Vítámvás *et al.* unpublished data). The identification of low Mr dehydrins in salinity-treated barley still remains to be resolved. The main obstacles to mass spectrometry identification probably lie in a high similarity of Y_nSK_m type dehydrin protein sequences.

Both qualitative and quantitative differences were found in the dehydrin protein accumulation between the barley cultivars. One protein of 82 kD corresponding to DHN5 and several low Mr proteins were detected in both the genotypes in response to the salinity. Qualitative differences in the range of accumulated dehydrin proteins can be explained either by different dehydrins accumulated or by a presence of different allelic variants for the same protein in the two genotypes. Isoforms of barley DHN4 protein with a different copy number of

hydrophilic Φ segments and thus different Mr values were reported by Choi *et al.* (1999). The dehydrin protein accumulation increased in both the cultivars under the high salt stress (300 mM NaCl) with a larger increase in Tadmor than in Amulet. Quantitative differences in the dehydrin accumulation between Amulet and Tadmor subjected to the same conditions correspond to our previous observations found in barley and wheat subjected to cold treatments (Vítámvás *et al.* 2010, Kosová *et al.* 2013) and confirm a potential utilization of a relative dehydrin accumulation as marker of stress tolerance in barley (Kosová *et al.* 2010).

An increased dehydrin content persisted in Tadmor after recovery. These results are in accordance with a slow dynamics of dehydrin protein degradation reported by Ohno *et al.* (2003) as well as by Vítámvás and Prášil (2008) in cold-treated winter wheat. The dynamics of dehydrin protein degradation is significantly slower than of transcript degradation (Kobayashi *et al.* 2004). Since a correlation between a quantitative dehydrin accumulation and an acquired plant stress tolerance has been found in previous experiments (Kosová *et al.* 2010), dehydrin protein persistence after recovery may represent an adaptation preventing plant tissue damage upon cyclic changes in ambient conditions (*e.g.*, freeze-thaw cycles or periodic flooding with seawater).

Primary photosynthetic processes are adversely affected by stresses resulting in an increase in alternative pathways of absorbed energy utilization. Chlorophyll *a* fluorescence represents one way for monitoring the efficiency of primary photosynthetic reactions (Lichtenthaler and Rinderle 1987, Krause and Weis 1991). Determination of F_v/F_m values has been proven a useful tool for assessment of PS II damage in plants subjected to cold, drought, and salinity (Rizza *et al.* 2001). Under optimum conditions, F_v/F_m values range around 0.8; however, they significantly decrease under stress (Krause and Weis 1991). Under the high salt stress (300 mM NaCl), F_v/F_m ratio decreased in both the cultivars with a more profound decrease observed in Amulet than in Tadmor. The persistence of decreased F_v/F_m values in Amulet after recovery indicates an irreversible damage of its photosynthetic apparatus. In contrast, Tadmor was able to recover as indicated by an increase in F_v/F_m values. An impairment of photosynthetic processes in salt-treated plants has also been indicated indirectly in proteomic studies by a decrease in many photosynthesis and carbon assimilation-related enzymes, such as Rubisco subunits (Kim *et al.* 2005, Caruso *et al.* 2008).

An evaluation of all the parameters studied (WSD, ψ_s, proline, F_v/F_m, DHN) by PCA has revealed a differential response of Amulet and Tadmor plants to the salt treatments, especially to 300 mM NaCl, and a subsequent recovery. At 300 mM NaCl, Tadmor revealed a higher accumulation of protective compounds (proline, dehydrins) and a lower damage of photosynthetic apparatus, indicated by F_v/F_m, than Amulet. Moreover, Tadmor was able to recover crucial parameters for further plant growth and development, whereas Amulet failed to recover. The differential recovery of Amulet and Tadmor was indicated by the presence of the Tadmor variants in the vicinity of the control plants, whereas the Amulet variants remained in the vicinity of the stressed plants in the PCA plot (Fig. 2 Suppl.). It can thus be concluded that the Syrian landrace Tadmor is more tolerant to a high-salt stress than the Czech spring cultivar Amulet. A comparison of the recovery in the plants gradually treated by NaCl up to 300 mM with the plants exposed directly to 300 mM NaCl has revealed a better recovery ability in the gradually acclimated plants (Fig. 2 Suppl.). The results of the PCA analysis thus confirm the importance of a gradual acclimation for an acquisition of an enhanced stress tolerance (Levitt 1980, Thomashow 1999, Larcher 2003).

Conclusions: Quantitative differences were found between barley cv. Amulet and landrace Tadmor in response to salt with Tadmor exhibiting lower WSD and higher ψ_s, F_v/F_m, and dehydrin content compared to Amulet. Qualitative differences were found between salt-treated Amulet and Tadmor in the dehydrin protein relative accumulation. A 29 kD protein band was detected only in Amulet, whereas a 26 kD protein band was detected only in Tadmor. A differential ability of Amulet and Tadmor to recover after the high-salt stress was also found. Decreased ψ_s and F_v/F_m and increased WSD and proline content persisted in Amulet after the recovery treatment indicating its serious damage. In contrast, Tadmor was able to restore tissue hydration and photosynthesis as key parameters for further plant growth.

References

Battaglia, M., Olvera-Carrillo, Y., Garciarrubio, A., Campos, F., Covarrubias, A.A.: The enigmatic LEA proteins and other hydrophilins. - Plant Physiol. **148**: 6-24, 2008.

Bose, J., Rodrigo-Moreno, A., Shabala, S.: ROS homeostasis in halophytes in the context of salinity stress tolerance. - J. exp. Bot. **65**: 1241-1257, 2014.

Bravo, L.A., Close, T.J., Corcuera, L.J., Guy, C.L.: Characterization of an 80-kDa dehydrin-like protein in barley responsive to cold acclimation. - Physiol. Plant. **106**: 177-183, 1999.

Brini, F., Hanin, M., Lumbreras, V., Irar, S., Pages, M., Masmoudi, K.: Functional characterization of DHN-5, a dehydrin showing a differential phosphorylation pattern in two Tunisian durum wheat (*Triticum durum* Desf.) varieties

with marked differences in salt and drought tolerance. - Plant Sci. **172**: 20-28, 2007.

Caruso, G., Cavaliere, C., Guarino, C., Gubbiotti, R., Foglia, P., Lagana, A.: Identification of changes in *Triticum durum* L. leaf proteome in response to salt stress by two-dimensional electrophoresis and MALDI-TOF mass spectrometry. - Anal. bioanal. Chem. **391**: 381-390, 2008.

Chen, Z., Cuin, T.A., Zhou, M., Twomey, A., Naidu, B.P., Shabala, S.: Compatible solute accumulation and stress-mitigating effects in barley genotypes contrasting in their salt tolerance. - J. exp. Bot. **58**: 4245-4255, 2007.

Choi, D.W., Zhu, B., Close, T.J.: The barley (*Hordeum vulgare* L.) dehydrin multigene family: sequences, allele types, chromosome assignments, and expression characteristics of 11 *Dhn* genes of cv. Dicktoo. - Theor. appl. Genet. **98**: 1234-1247, 1999.

Colmer, T.D., Flowers, T.J., Munns, R.: Use of wild relatives to improve salt tolerance in wheat. - J. exp. Bot. **57**: 1059-1078, 2006.

De Lacerda, C.F., Cambraia, J., Oliva, M.A., Ruiz, H.A., Prisco, J.T.: Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. - Environ. exp. Bot. **49**: 107-120, 2003.

Flowers, T.J.: Improving crop salt tolerance. - J. exp. Bot. **55**: 307-319, 2004.

Hong, Z., Lakkineni, K., Zhang, Z., Verma, D.P.S.: Removal of feedback inhibition of Δ^1-pyrroline-5-carboxylate synthetase results in increased proline accumualtion and protection of plants from osmotic stress. - Plant Physiol. **122**: 1129-1136, 2000.

Islam, S., Malik, A.I., Islam, A.K.M.R., Colmer, T.D.: Salt-tolerance in a *Hordeum marinum-Triticum aestivum* amphiploid, and its parents. - J. exp. Bot. **58**: 1219-1229, 2007.

Jiménez-Bremont, J.F., Becerra-Flora, A., Hernández-Lucero, E., Rodríguez-Kessler, M., Acosta-Gallegos, J.A., Ramírez-Pimentel, J.G.: Proline accumulation in two bean cultivars under salt stress and the effect of polyamines and ornithine. - Biol. Plant. **50**: 763-766, 2006.

Kim, D.W., Rakwal, R., Agrawal, G.K., Jung, Y.H., Shibato, J., Jwa, N.S., Iwahashi, Y., Iwahashi, H., Kim, D.H., Shim, I.S., Usui, K.: A hydroponic rice seedling culture model system for investigating proteome of salt stress in rice leaf. - Electrophoresis **26**: 4521-4539, 2005.

Kishor, P., Hong, Z., Miao, G.H., Hu, C., Verma, D.: Overexpression of [delta]-pyrroline-5-carboxylate synthatase increases proline production and confers osmotolerance in transgenic plants. - Plant Physiol. **108**: 1387-1394, 1995.

Kobayashi, F., Takumi, S., Nakata, M., Ohno, R., Nakamura, T., Nakamura, C.: Comparative study of the expression profiles of the *Cor*/*Lea* gene family in two wheat cultivars with contrasting levels of freezing tolerance. - Physiol. Plant. **120**: 585-594, 2004.

Kosová, K., Holková, L., Prášil, I.T., Prášilová, P., Bradáčová, M., Vítámvás, P., Čapková, V.: Expression of dehydrin 5 during the development of frost tolerance in barley (*Hordeum vulgare*). - J. plant Physiol. **165**: 1142-1151, 2008.

Kosová, K., Prášil, I.T., Vítámvás, P.: Role of dehydrins in plant stress response. - In Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. 3rd Ed. Pp. 239-285. CRC Press, Taylor & Francis, Boca Raton 2010.

Kosová, K., Vítámvás, P., Prášilová, P., Prášil, I.T.: Accumulation of WCS120 and DHN5 proteins in differently frost-tolerant wheat and barley cultivars grown under a broad temperature scale. - Biol Plant. **57**: 105-112, 2013.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: the basics. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. - Nature **277**: 680-685, 1970.

Larcher, W.: Physiological Plant Ecology. 4th Ed. - Springer-Verlag, Berlin - Heidelberg 2003.

Lawlor, D.W.: Genetic engineering to improve plant performance under drought: physiological evaluation of achievements, limitations, and possibilities. - J. exp. Bot. **64**: 83-108, 2013.

Levitt, J.: Responses of plants to environmental stress. Chilling, freezing and high temperature stresses. 2nd Ed. - Academic Press, New York 1980.

Lichtenthaler, H.K., Rinderle, U.: The role of chlorophyll fluorescence in the detection of stress conditions in plants. - Crit. Rev. anal. Chem. **19**: 29-85, 1988.

Munns, R.: Comparative physiology of salt and water stress. - Plant Cell Environ. **25**: 239-250, 2002.

Munns, R.: Genes and salt tolerance: bringing them together. - New Phytol. **167**: 645-663, 2005.

Munns, R., Tester, M.: Mechanisms of salinity tolerance. - Annu. Rev. Plant Biol. **59**: 651-681, 2008.

Ohno, R., Takumi, S., Nakamura, C.: Kinetics of transcript and protein accumulation of a low-molecular weight wheat LEA-D11 dehydrin in response to low temperature. - J. Plant Physiol. **160**: 193-200, 2003.

Ozturk, Z.N., Talamé, V., Deyholos, M., Michalowski, C.B., Galbraith, D.W., Gozukirmizi, N., Tuberosa, R., Bohnert, H.J.: Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley. - Plant mol. Biol. **48**: 551-573, 2002.

Prášil, I.T., Prášilová, P., Pánková, K.: The relationship between vernalization requirement and frost tolerance in substitution lines of wheat. - Biol. Plant. **49**: 195-200, 2005.

Rasoulnia, A., Bihamta, M.R., Peyghambari, S.A., Alizadeh, H., Rahnama, A.: Proteomic response of barley leaves to salinity. - Mol. Biol. Rep. **38**: 5055-5063, 2011.

Rizza, F., Pagani, D., Stanca, A.M., Cattivelli, L.: Use of chlorophyll fluorescence to evaluate the cold acclimation and freezing tolerance of winter and spring oats. - Plant Breed. **120**: 389-396, 2001.

Rorat, T.: Plant dehydrins – tissue location, structure and function. - Cell. mol. Biol. Lett. **11**: 536-556, 2006.

Serraj, R., Sinclair, T.R.: Osmolyte accumulation: can it really help increase crop yield under drought conditions? - Plant Cell Environ. **25**: 333-341, 2002.

Slavík, B.: Relationship between the osmotic potential of cell sap and the water saturation deficit during the wilting of leaf tissue. - Biol. Plant. **5**: 258-264, 1963.

Suprunova, T., Krugman, T., Fahima, T., Chen, G., Shams, I., Korol, A., Nevo, E.: Differential expression of dehydrin genes in wild barley, *Hordeum spontaneum*, associated with resistance to water deficit. - Plant Cell Environ. **27**: 1297-1308, 2004.

Thomashow, M.F.: Plant cold acclimation. Freezing tolerance genes and regulatory mechanisms. - Annu. Rev. plant Physiol. Plant mol. Biol. **50**: 571-599, 1999.

Tommasini, L., Svensson, J.T., Rodriguez, E.M., Wahid, A., Malatrasi, M., Kato, K., Wanamaker, S., Resnik, J., Close, T.J.: Dehydrin gene expression provides an indicator of low temperature and drought stress: transcriptome-based analysis of barley (*Hordeum vulgare* L.). - Funct. integr. Genomics **8**: 387-405, 2008.

Van Zee, K., Chen, F.Q., Hayes, P.M., Close, TJ., Chen, T.H.H.: Cold-specific induction of a dehydrin gene family member in barley. - Plant Physiol. **108**: 1233-1239, 1995.

Vítámvás, P., Kosová, K., Prášilová, P., Prášil, I.T.: Accumulation of WCS120 protein in wheat cultivars grown at 9 °C or 17 °C in relation to their winter survival. - Plant Breed. **129**: 611-616, 2010.

Vítámvás, P., Prášil, I.T.: WCS120 protein family and frost tolerance during cold acclimation, deacclimation and reacclimation of winter wheat. - Plant Physiol. Biochem. **46**: 970-976, 2008.

Voetberg, G., Stewart, C.R.: Steady state proline levels in salt-shocked barley leaves. - Plant Physiol. **76**: 567-570, 1984.

Walia, H., Wilson, C., Ismail, A.M., Close, T.J., Cui, X.: Comparing genomic expression patterns across plant species reveals highly diverged transcriptional dynamics in response to salt stress. - BMC Genomics **10**: 398, 2009.

Yamaguchi-Shinozaki, K., Shinozaki, K.: Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. - Annu. Rev. Plant Biol. **57**: 781-803, 2006.

Zhang, J., Nguyen, H.T., Blum, A.: Genetic analysis of osmotic adjustment in crop plants. - J. exp. Bot. **50**: 291-302, 1999.

Zhu, B., Choi, D.W., Fenton, R., Close, T.J.: Expression of the barley dehydrin multigene family and the development of freezing tolerance. - Mol. gen. Genet. **264**: 145-153, 2000.

Zhu, J.K.: Salt and drought stress signal transduction in plants. - Annu. Rev. Plant Biol. **53**: 247-273, 2002.