

Influence of salt stress on growth and frost resistance of three winter cereals

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Received February 22, 2014; accepted November 4, 2014

A b s t r a c t. This paper presents results of a study on the influence of 0-150 mmol NaCl dm⁻³ Hoagland solution on growth, chlorophyll content, photosynthesis and frost resistance of seedlings of three winter cereals: wheat – *cv.* Almari, rye – *cv.* Amilo, and triticale – *cv.* Tornado. Sodium chloride at 25 mmol dm⁻³ caused better growth of wheat shoots and roots, both of fresh and dry matter. Higher concentrations of NaCl in the medium decreased the biomass of the tested seedlings. The influence of NaCl on the chlorophyll content in the seedlings varied. The conductometry method showed that the resistance of the cell walls of wheat and rye to low temperature decreased in the presence of NaCl in the growth medium. Luminescence has shown that seedlings that grew in NaCl-containing medium indicated an impediment of electron flow at a lower temperature than the control plants.

K e y w o r d s: NaCl, frost resistance, growth, winter cereals

INTRODUCTION

Plants that grow in the natural environment are exposed to various unfavourable factors, which modify the intensity or disturb their growth, thereby causing temporary or irreversible destabilization of the organism.

The reaction of plants to a single stress factor, such as low temperature (Beck *et al.*, 2007; Mahajan and Tuteja, 2005) or salinity (Chinnusamy *et al.*, 2005; Flowers, 2004; Mohammadi *et al.*, 2012; Munns, 2002), is fairly well recognized. The response of cultivated plants to stress caused by a number of stress factors factors is a more complex issue, as in natural conditions, plants are usually exposed to multistress. Since the area of saline soils increases in the world (Arzani, 2008; Munns, 2002) and plants, for example

winter rye, are exposed to both salt and low temperature, studies on the effect of combined stress factors seem to be of great importance.

As far as winter plants are concerned, their yield depends to a great extent on winter hardiness and frost resistance, which are conditioned by both genetic factors and the environment (Pocock *et al.*, 2001; Starck *et al.*, 1995, Veisz *et al.*, 2001).

Climatic and soil conditions can modify plant frost resistance by modifying their frost hardening. Thus, it can be assumed that salinity of the growth medium, which is one of the important factors limiting plant cultivation, may affect plant frost resistance, providing the intensity of salt stress would not be overly intense and the plants would not perish.

Knowledge of plant response to various stresses, particularly to multistresses, is of great scientific and practical value. Selection of species and cultivars that show resistance to stress factors can lead to an increase in areas suitable for plant cultivation. Moreover, it can promote cultivation of plant forms that will possess mechanisms increasing their stress resistance.

The aim of this study was to assess the influence of growth medium salinity (NaCl) on growth and frost resistance of some winter cereal cultivars. The study also aimed at an analysis of these effects, assuming that moderate salt stress might promote winter hardiness.

MATERIAL AND METHODS

The experiments were conducted in controlled conditions, using the Hoagland solution as a growth medium for winter cereals: wheat (*Triticum aestivum* L.) *cv.* Almari, rye

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(*Secale cereale* L.) cv. Amilo, and triticale (*Triticosecale* Wittm.) cv. Tornado. Sodium chloride solutions were mixed with the Hoagland solution 1:1 to obtain NaCl concentrations of 25, 50, 75, 100, and 150 mmol dm⁻³. Hoagland solution diluted with distilled water 1:1 served as a control medium.

Kernels of the tested cereals were placed in the 'Szmal' germination apparatus (Kospin, Poddębice, Poland), 100 kernels per one, and then transferred into containers with Hoagland solution with various NaCl concentrations. The samples were kept in a microphytotron at 20°C, photosynthetic photon flux density (PPFD) 200 μmol m⁻² s⁻¹, day/night 12 h/12 h. Beginning with the third day, the temperature was decreased by 2°C per day to 10°C. The plants were kept in these conditions for 28 days.

The cereal kernels were placed in distilled water in Petri dishes for one day. The next day, 20 kernels were transferred onto wet filter paper (5x22.5 cm), 1 cm off the upper edge, covered with a strip of wet filter paper, and rolled into tubes. These tubes were then put upright in glass dishes (three in one dish) containing a 1-cm layer of distilled water. 24 h later, the water was replaced by NaCl solutions, and the samples were placed in a miniphytotron (20°C, PPFD 200 μmol m⁻² s⁻¹, day/night 12 h/12 h). The temperature was lowered by 3°C per day to achieve 10°C and 14 days later the process of hardening began. The PPFD was decreased to 60 μmol m⁻² s⁻¹, and the day/night period to 8 h/16 h. Simultaneously, the temperature was successively lowered with the rate 2°C/24 h to 2°C. After 14 days, the solutions were removed from the dishes, and the plants remaining in the filter-paper tubes were moistened every day with the proper NaCl – Hoagland solutions, which lasted for 14 days.

The fresh and dry matter of the seedlings were analyzed by weight, with an accuracy of 0.0001 g. The plants were dried for 12 h at 105°C. An average weight of 1 seedling was calculated.

The portable photosynthesis meter (LCA-4 (ADC Bioscientific Ltd. Great Britain)) was used to measure the intensity of photosynthesis.

The optical density of leaf mesophyll was measured by SPAD-502 (Minolta Co. Ltd, Japan).

The result of these measurements was displayed digitally as the so-called SPAD units. Its value is proportional to the content of chlorophyll in the analyzed leaf surface (6 mm²) (Monje and Bugbee, 1992).

To assess the cell wall resistance to low temperature, a modified conductometric method was applied (Brzóstowicz and Prokowski, 2003). Shoots of each seedling were cut off at the seed, rinsed twice with distilled water, and placed in moist test tubes covered with cotton balls. These tubes (10 in each treatment) were placed in test tube holders; 5 such batches were transferred into 5 freezers. The control batch was kept for 24 h at 5 ± 1°C. The remaining 4 batches were kept in the same conditions for only 15 min and next the temperature was lowered by 1°C per 5 min to achieve the desired temperature (-14, -16, -18, and -20°C). The temperature was different for the various plant species

in order to produce damage below or above 50%. After 2 h of freezing the seedlings, the temperature was increased by 1°C per 5 min to reach +5°C. 12 h later, 7 ml distilled water with a temperature of 5 °C was added to each test tube. After 4 h (including 30-min shaking), the conductivity of the consecutive solutions was measured using the CPC-551 conductometer (Elmetron, Poland). The solution was placed back into the test tube with the seedlings. The tested plants were then kept for 16 h in a freezer at -30°C to achieve total frost kill. After quick thawing and warming to room temperature, 4 h after removal (including 30 min shaking), electrical conductance was measured again (maximum leakage of the electrolyte). The damage index 'ID' (Flint *et al.*, 1967) for the given temperature of chilling was then calculated. This index expresses the percentage of damage of the tested object in relation to maximal damage that can occur after total destruction by low temperature. The frost resistance index in this method is the temperature t_{50} which is read from the diagram of the dependence of the damage index on the freezing temperature. The lower the t_{50} the greater is the frost resistance of the cell walls. The diagram facilitated the assessment of the precision of the t_{50} assay after the absolute error calculation of the damage index had been done with the total differential method.

The conductometric method of the assessment of frost resistance was applied to hardened plants subjected to various salinity of the growth medium. Each experiment was repeated 10 times.

Parts of the first leaf (2 cm long) were taken at a distance 2 cm from the tip. The leaves were placed on strips of moist filter paper and kept in Petri dishes in a freezer at 5°C, PPFD 200 μmol m⁻² s⁻¹. Delayed luminescence (IDL) of chlorophyll was measured during the period of temperature dropping with a speed of 1°C per 1 min, from 0 to -20°C, using a test stand developed by Brzóstowicz (1993), and modified by Brzóstowicz (2003) at the Department of Physics, Agricultural University in Szczecin. Previous studies (Brzóstowicz, 1990) had shown that t_m (the temperature at which delayed luminescence was at its maximum), was well suited for the assessment of the sensitivity of the photosynthetic apparatus of plants to low temperature. The t_m index informs about the temperature at which the electron transport between Q_A and Q_B was blocked at the acceptor site of photosystem II. A lower value of t_m at constant measurement conditions indicates higher resistance of initial reactions within photosystem II to low temperature, hence higher potential of frost resistance.

These assays were conducted with hardened plants growing in a medium with a varied NaCl content, with 5 replicates for each object.

The statistical analysis was done using the Statistica program, version 6.0. The significance of the influence of the NaCl concentration on the analyzed features was assessed by one-factorial analysis of variance, Tukey test, $p < 0.05$.

On the basis of one- and two-factorial analysis of variance using Tukey test $p < 0.05$, homogeneous groups were distinguished.

RESULTS AND DISCUSSION

Tables 1 and 2 show the dependence of fresh and dry matter of wheat, rye, and triticale seedlings on the content of NaCl in the growth medium. An appropriate concentration of salt was chosen to make the growth and development of a majority of plants possible, despite the salinity of the medium, and to achieve major differentiation of the biometric features of the plants. It was found that with growing salinity the plants produced less biomass. Salinity of 150 mmol NaCl dm⁻³ caused the greatest inhibition of growth, both in roots and shoots. Wheat and triticale growing in the medium with the highest NaCl concentration produced biomass of only 50% of that of the control plants, whereas rye biomass decreased at that concentration by 80%. The growth of roots was more affected than that of shoots: both fresh and dry matter of seedlings grown in a medium containing 150 mmol NaCl in 1 litre produced

80% less biomass than the control plants. It can be assumed that this was due to disturbances in water uptake, ion balance, and toxicity of sodium and chloride ions (Chinnusamy *et al.*, 2005; Starck *et al.*, 1995).

A stimulating effect of NaCl was found only in the growth of wheat grown in the medium containing 25 mmol NaCl dm⁻³. It increased significantly the fresh matter of shoots and dry matter of roots. In earlier investigations conducted on wheat (Matuszak and Brzóstowicz, 2003), a stimulating influence of lower NaCl concentrations on the germination capability, the length of leaf, and fresh and dry matter of overground parts of seedlings was also found. Furthermore, an influence of NaCl on the increase in cell membrane permeability, both in unhardened and hardened plants, was confirmed. An increase in low temperature resistance of the photosynthetic apparatus under the influence of NaCl was also found.

According to many authors, a majority of cultivated plants are typical glycophytes with low salt tolerance (Starck *et al.*, 1995). Cereals exhibit moderate tolerance to salinity; barley has the greatest tolerance, wheat displays

Table 1. Effect of NaCl on fresh and dry matter of shoots of wheat, rye, and triticale seedlings

NaCl (mmol dm ⁻³)	Fresh matter (mg)			Dry matter (mg)		
	wheat	rye	triticale	wheat	rye	triticale
0	227.71 c A	400.38 d B	342.40 c AB	40.21 cd A	57.52 b A	55.67 b A
25	274.76 d A	250.09 c A	308.1 bc A	46.15 d A	44.15 ab A	55.56 ab A
50	225.13 c A	204.8 bc A	316.24 bc B	41.90 cd A	43.51 ab A	47.07 ab A
75	166.48 b A	171.3 ab A	213.05 ab B	32.42 b A	35.35 ab A	37.80 ab A
100	167.62 b B	137.80 ab A	205.54 ab C	35.13 bc AB	30.09 a A	39.67 ab B
150	113.37 a B	68.86 a A	127.00 a B	24.32 a A	22.03 a A	25.44 a A

Deficiency of significant differences ($p > 0.05$): a, b, c – within the species; A, B, C – between the species.

Table 2. Influence of NaCl on fresh and dry matter of roots of wheat, rye, and triticale

NaCl (mmol dm ⁻³)	Fresh matter (mg)			Dry matter (mg)		
	wheat	rye	triticale	wheat	rye	triticale
0	275.21 bc AB	155.22 c A	334.39 c B	22.41 c A	13.55 b A	22.54 b A
25	324.49 c B	119.21 bc A	285.13 bc B	26.97 d B	13.52 b A	23.13 b B
50	258.28 b B	117.40 bc A	314.24 bc B	22.00 c B	12.97 b A	18.80 ab B
75	111.24 a A	103.42 bc A	161.05 ab B	13.43 b A	10.77 ab A	16.16 ab B
100	115.93 a B	64.88 ab A	119.54 a B	12.16 b B	6.14 ab A	13.52 a B
150	64.38 a B	20.39 a A	76.58 a B	7.52 a B	2.60 a A	8.74 a B

Explanations as in Table 1.

lower tolerance, and rye and oats are relatively susceptible to salinity (Munns, 2002). Based on this study, it was hard to determine which of the tested species were more and which less susceptible to salinity. Investigations of the influence of NaCl on the fresh and dry matter of overground parts of seedlings (Table 1) revealed minor differences between species grown in the control solution and the NaCl solution. It was observed that triticale seedlings were characterized by the highest levels of fresh matter of the overground parts in comparison with the other tested species. In the analysis of the influence of NaCl on fresh and dry matter of roots (Table 2), it was found that rye seedlings produced the lowest biomass. On the contrary, minor differences between wheat and triticale seedlings grown on the control and NaCl solutions in terms of fresh and dry matter were observed. The reaction of plants depends *eg* on the growth stage (Munns, 2002), kind of salt, and external factors (Shannon and Grieve, 1999). Furthermore, there are differences between not only species but also cultivars of one species (Munns, 2002).

The impact of NaCl on the content of chlorophyll in the first leaves of wheat, rye, and triticale is shown in Fig. 1. Many authors have found that plants react to salinity by a reduced content of chlorophyll in leaves (Muranaka *et al.*, 2002). This may be due to increased activity of chlorophyllases and accumulation of salt ions. In our study, a significantly higher chlorophyll content than in the control seedlings was found only in triticale which had grown in a medium containing 150 mmol NaCl dm⁻³. The other plants either showed no reaction to the presence of NaCl in the medium or had a lower content of chlorophyll. These findings indicate that plants react to salt contained in the medium in various manners. It was observed, comparing the reaction

of the particular species to salt stress, that the triticale seedlings were indeed characterized by the greatest chlorophyll content in the first leaf.

Figure 2 presents the influence of NaCl on the intensity of photosynthesis. The presence of NaCl in the growth medium caused a decrease in photosynthesis intensity, which was greater in media with increasing concentrations of salt. Several authors have found that this phenomenon is caused by *eg* a lower chlorophyll content, closure of stomata (Muranaka *et al.*, 2002; Starck *et al.*, 1995), lower activity of RuBP-carboxylase (Flexas *et al.*, 2004), disturbance in electron transport, and disorder in photosystem II (Muranaka *et al.*, 2002). Furthermore, salt stress decreases biomass production affecting parameters connected with gas exchange and transpiration (Kalaji and Pietkiewicz, 1993), which was confirmed in this study. In their investigations of the impact of salinity on growth, photosynthesis, water potential, and leaf temperature of wheat seedlings (*cv.* Almari), Matuszak *et al.* (2004) found a decrease in photosynthesis intensity with increasing NaCl concentration, lowered water potential in leaves and, on the basis of thermal pictures analysis, growth of leaf temperature. In this case, the excess of salinity in the water solution reduced cell hydration and, in consequence, induced a fall in turgor. The fall inhibited the growth and caused a temperature increase in wheat seedling leaves.

The dependence of the average temperature t_m at which the maximum delayed luminescence (IDL) was the lowest on the concentration of NaCl in the medium is presented in Fig. 3. In the case of triticale, results showing the effect of NaCl at concentrations up to 75 mmol NaCl dm⁻³ are only presented, due to the loss of samples. The reduction of the t_m values with the increasing salt content in the medium

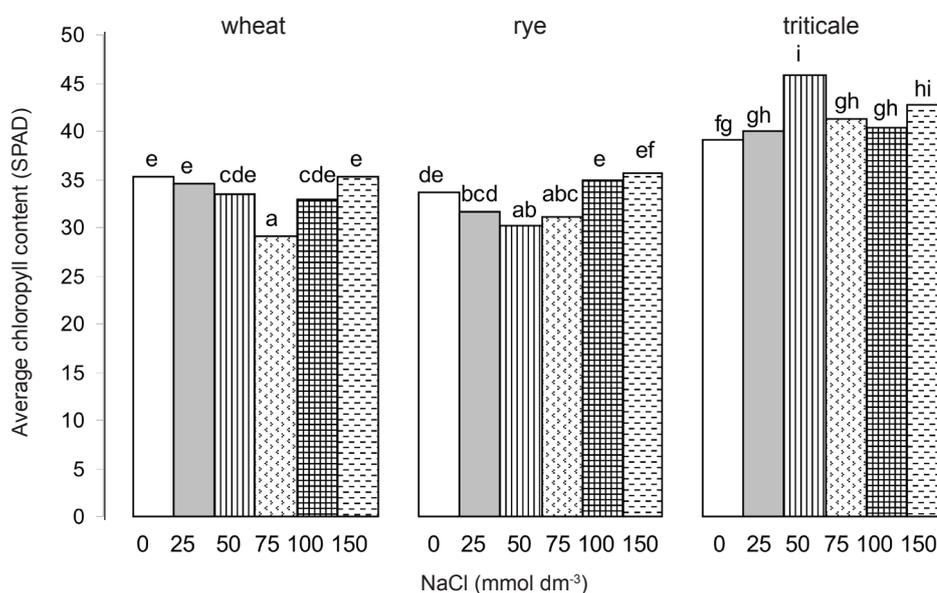


Fig. 1. Dependence of the chlorophyll content in the first leaf of wheat *cv.* Almari, rye *cv.* Amilo, and triticale *cv.* Tornado on the NaCl concentration. Numbers with the same letter mean that the differences are not significant at the level $p < 0.05$. Tukey test – two-factorial analysis of variance.

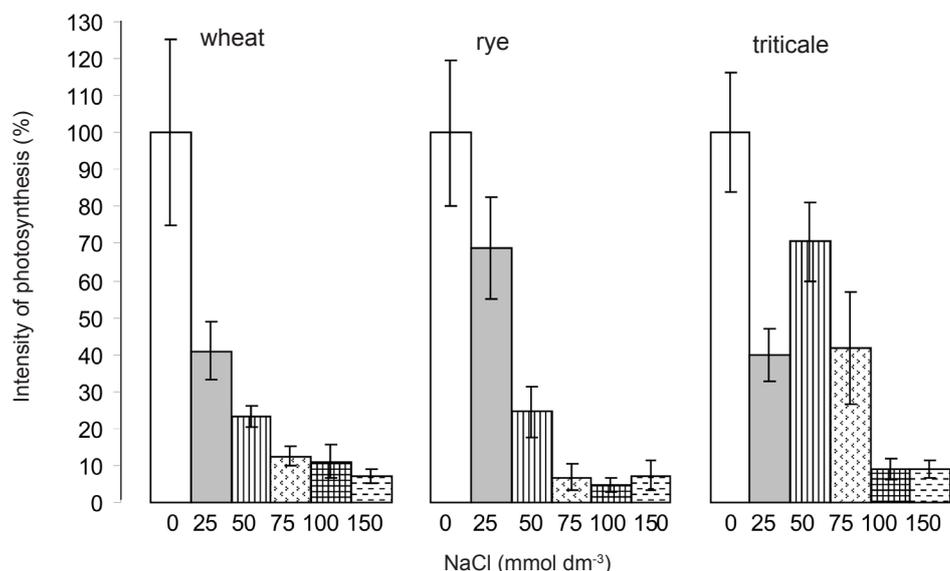


Fig. 2. Influence of NaCl on photosynthesis intensity in wheat *cv.* Almari, rye *cv.* Amilo, and triticale *cv.* Tornado (100% = photosynthesis of control plants). Mean values of 6 replicates \pm standard deviation (SD).

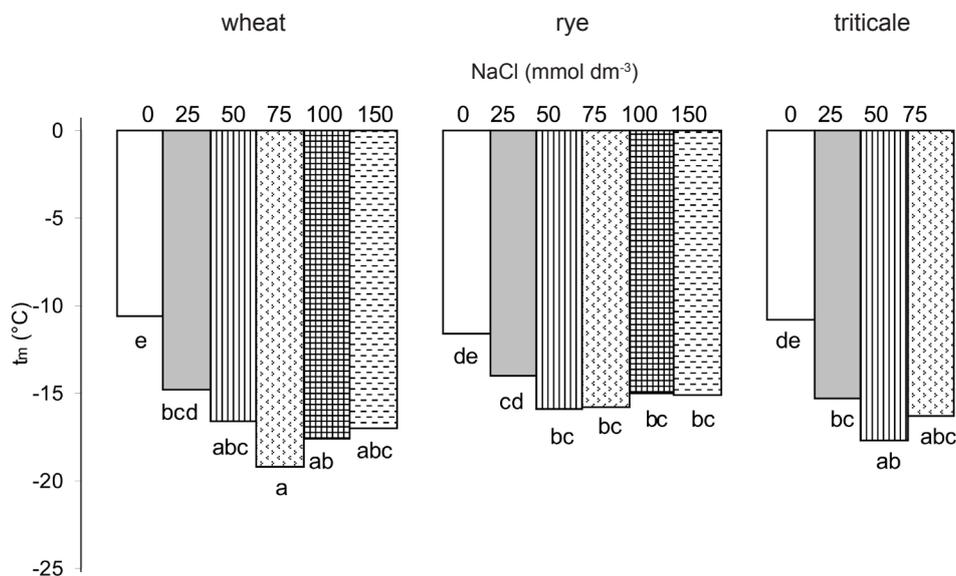


Fig. 3. Dependence of temperature (t_m) at which maximum delayed luminescence (IDL) was reported on the NaCl concentration in wheat, rye and triticale hardened against low temperature. Explanations as in Fig. 1.

indicates a shift of the IDL maximum toward lower temperature. Besides, no significant differences in the value of the t_m indicator between the tested species grown in the control solution and the NaCl solutions were observed. Brzóstowicz (1990), who studied the effect of low temperature on the intensity of the secondary component of leaf delayed luminescence, found that changes in this phenomenon (size and location of the maximum) depend on the temperature at which the electron transport between the Q_A and Q_B acceptors in photosystem II is blocked and on the potential gradient generated by hydrogen ion transport through the thylakoid membrane in a reaction coupled with photo-

synthetic electron transport. In hardened plants, the electron transport and hydrogen ion transport can take place quite efficiently at a lower temperature than in non-hardened plants.

The results of this study have shown that the presence of NaCl in the medium blocks electron transport at the acceptor site of photosystem II at a lower temperature. Therefore, the resistance of the electron transport is higher than in the control plants. It can also be assumed that the lower values of t_m may be caused by efficient accumulation and compartmentation of toxic ions in the vacuole, which further leads to decreased transport of Na^+ and Cl^- into chloroplasts, hence causing little damage (Chinnusamy *et al.*,

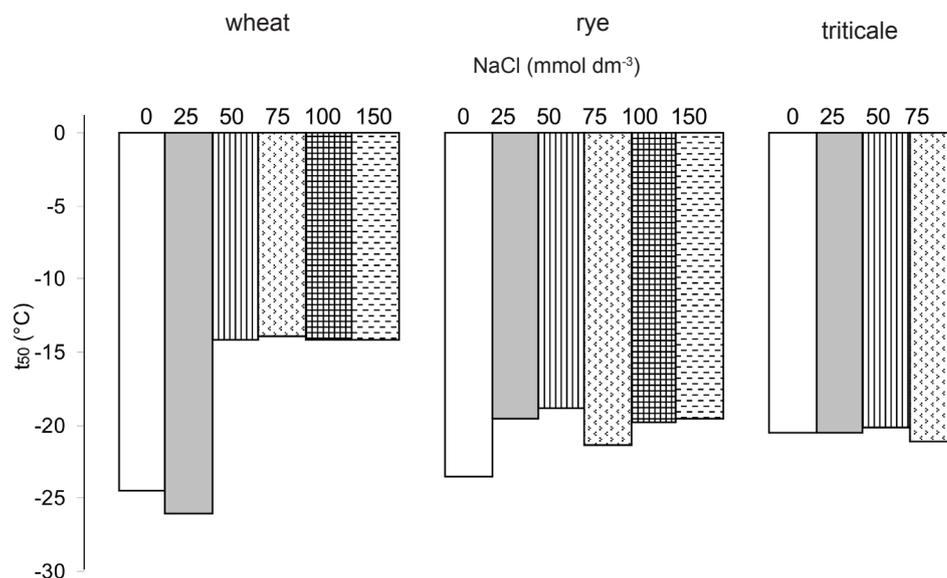


Fig. 4. Dependence of temperature t_{50} (50% damage) on the concentration of NaCl in the growth medium for wheat, rye, and triticale hardened for low temperature (standard deviation did not exceed 1.5°C).

2005). The increased resistance of electron transport to low temperature may also be due to the synergetic activity of both the low temperature and salinity. Under the influence of salinity and hardening, plants synthesize factors protecting the photosynthetic apparatus (Aticia *et al.*, 2003; Starck *et al.*, 1995) and maintain the osmotic balance. This changes the chemical composition and properties of cell walls (thylakoid walls as well) – the amount of non-saturated acids increases, which causes maintenance of high activity of enzymes in the walls, particularly of the ATP-ases (Starck *et al.*, 1995). Moreover, with increasing salinity the temperature of crystallization decreases and the process of intracellular freezing is delayed. These changes are correlated with an increased concentration of the cell liquid. These processes are involved in energy transfer, which in turn affects the activity of photosynthesis. The activities mentioned above and the decreased fluidity of membranes can increase the potential gradient in plants exposed to low stress temperature, which can be detected as a greater maximum of delayed luminescence intensity (Brzóstowicz, 2003).

The data presented in Fig. 4 show the dependence of the t_{50} index on the concentration of NaCl in the growth medium for wheat, rye, and triticale which had been hardened against frost. With the exception of the triticale seedlings, whose cell walls did not react to the presence of NaCl in the medium, the two other species, particularly wheat, exhibited increased permeability of cell walls caused by the higher content of NaCl in the growth medium. Comparison of temperature values t_{50} determined for the particular species revealed that wheat seedlings grown on NaCl solution at 25 mmol dm^{-3} were characterized by a lower t_{50} temperature value, but those grown in the presence of higher NaCl concentrations displayed the highest temperature values. These facts imply greater sensitivity to low temperature of the cell membrane in wheat seedlings than in

rye and triticale membranes. An increase in t_{50} (temperature that induced a 50% leak of electrolytes from the damaged tissue) indicated that the cell walls of rye and wheat were more sensitive to low temperature when they were grown in a NaCl containing medium. Changes in the cell wall permeability can be caused by both the increased salinity and low temperature. Salinity leads *eg* to depolarization of cell walls and changes in the chemical composition of lipids (Mansour and Salama, 2004) and opens ion and water channels, which causes water outflow with ions. Under the low temperature stress, dehydration of cells caused by crystallization of extracellular water leads to alterations in the cell wall structure and formation of lipid areas lacking protein molecules in the cell wall. As a result of low temperature, a thermotropic change in the lipid phase occurs and phase transition of the cell membrane takes place – the liquid crystal phase changes into a crystalline one (gel) (Badea and Basu, 2009). These changes often lead to denaturation and dissociation of proteins, *eg* enzymatic proteins, which leads to changes in the activity of numerous enzymes associated with cell walls (*eg* ATP-ase, which is responsible for the transport of many substances and ions). Cell walls of damaged cells cease to be semipermeable (Badea and Basu, 2009; Starck *et al.*, 1995).

The results obtained with the conductometric method indicate that NaCl affects strongly the permeability of the cell walls of the tested seedlings. In this case, the negative influence of accumulation of two stress factors caused the damage. Such injuries of the cell walls were observed in wheat and rye, but not in triticale. On the other hand, luminescence has proved that the presence of NaCl in the growth medium increased the resistance of the photosynthetic apparatus, because the electron transport was blocked at a lower temperature than in control plants.

An increase in frost resistance of spinach along with an increase in the NaCl concentration in the hydroponics culture medium was also observed by Schmidt *et al.* (1986). Both the osmolality of the leaf sap and frost resistance of the leaves were linearly correlated with the salt concentration in the hydroponics culture medium. In their investigations on the cauliflower, Fuller and Eed (2003) found that the most salt-resistant plants were also more frost tolerant.

At the beginning of this experiment, it was assumed that moderate salt stress might promote frost hardening, but it was difficult to predict to what extent. In literature, the phenomenon of cross-tolerance, when a plant exposed to one stress factor may become more tolerant to another factor, has been known for a long time (Płażek and Žur, 2003; Starck *et al.*, 1995; Titov *et al.*, 2003). Acquiring tolerance to one stress often decreases sensitivity for other stress factors. For example, Lichtenthaler (1996) reports that small doses of stress factors (*ie* deficiency of water) cause plant hardening, thereby lowering the sensitivity to other stress factors such as cold or salinity. Long-lasting effects of low concentrations of disadvantageous factors sometimes lead to growth at tolerance and stable acclimatization or even plant adaptation. Those processes occur in anatomical, morphological, biochemical, and physiological modifications both in photosynthesis and in donor-acceptor reactions. The results of biometric assays and assessment of frost resistance have shown that seedlings that developed larger shoots and roots if grown in a medium containing a low amount of NaCl (25 mmol dm⁻³) and those that had not suffered under the influence of salt exhibited increased resistance of the photosynthetic apparatus to low temperature. This was indicated by a lower t_m value in plants that grew in a medium with higher salinity, as compared with the control plants. On the other hand, damage done to cell walls (assessed by conductometry) indicated serious changes in cell wall permeability.

As shown by the presented investigations and professional literature reports, plant resistance to simultaneous salt and frost temperature stress depends on the plant species.

The luminescence measurements used in the analyses of the leaves of the seedlings provide additional information about the plant resistance or sensitivity to stress, especially about the sensitivity of the electron transport in the initial photosynthetic reactions.

Complex measurements using different methods provide more complete information about the physiological condition of plants exposed to stress factors.

The enhanced resistance to low temperature shown by seedlings growing in the saline medium was probably due to the very low osmotic potential of the cell liquid caused by both ion accumulation and active osmo-regulation. This influences further water uptake and protects the cell structures. The increased concentration of cell liquid delays crystallization of water.

CONCLUSIONS

1. The highest salt content in the growth medium (150 mmol NaCl dm⁻³) significantly decreased the weight of both the roots and shoots of the tested cereal: wheat, rye, triticale in comparison to control solution.
2. Low salinity of the Hoagland solution (25 mmol dm⁻³) stimulated the growth (fresh and dry matter) of wheat *cv.* Almari seedlings.
3. A majority of the cereals reacted to increasing salinity of the medium by reduced growth of shoots and roots, and the individual reactions varied.
4. The t_{50} index assessed by conductometry indicated that cell walls of wheat and rye growing in the saline medium were more susceptible to low temperature than those of triticale (grown in the same conditions).
5. The lowering of the temperature at which delayed luminescence was at its maximum following an increase in the NaCl content in the medium indicates blockage of electron transport at the acceptor site of photosystem II at a lower temperature.

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