

THE INFLUENCE OF HIGH NATRIUM AND CHLORINE ION CONCENTRATION ON PHYSIOLOGICAL RESPONSES OF VARIOUS SPRING BARLEY VARIETIES

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ABSTRACT

The effect of salinity on physiological processes of spring barley was monitored in varieties Amulet, Jersey, Krona, Malz, Norimberk and Valticky. The plants were grown under controlled light and temperature conditions in an air-conditioned chamber as a hydroponic culture in Knop's nutrient solution. The conditions of salinity in the nutrient solution were induced by NaCl at concentration levels of 0.06M, 0.15M and 0.25M. After 5-day exposure to the stress conditions, the physiological characteristics of the plants were measured at the stage of a fully developed 3rd leaf. The content of Na⁺ increased and at the same time, the K⁺ content in the leaves decreased in all the varieties. Also, depression in the growth of leaves occurred in all the examined varieties. The highest capability of osmotic adaptation was identified in the Norimberk, Malz and Jersey varieties. With respect to water use efficiency, the Amulet, Malz and Krona varieties demonstrated better water management under the salinity conditions than other varieties. With respect to changes in the growth of leaves and roots, the growth of leaves in the Amulet and Malz varieties was more inhibited by salinity than the growth of roots. In the other varieties, the response was the opposite.

Key words: spring barley, salinity stress, osmotic potential, water potential, photosynthesis, transpiration, growth.

INTRODUCTION

Soil salinity is an important worldwide problem mainly in arid and semiarid regions (Khosravinejad et al., 2009a). El-Hendawy et al. (2007) show, that the issue of soil salinity is addressed by 35–50 % of the world population from approximately 80 countries.

As Munns (2002) states, harmful toxic ions accumulate mainly in older leaves. The accumulation of the toxic ions of salt can even ultimately lead to the death of organs and the whole plants. The plants accumulate the harmful ions Na⁺ and Cl⁻ in vacuoles in order to minimise cytotoxicity. This can be an important part of the tolerance of cereals to salinity.

Based on the results of many papers, it can be said that the degree of tolerance to the toxic effect of ions Na⁺ and Cl⁻ varies according to plant species. It is true that barley is mentioned as a plant sensitive to salinity but it is a plant tolerating minor salinity. Wheat is capable of growing on soils containing up to 0.5 % of salt while barley tolerates even a double of that amount of salt in soil (Ali et al., 2001).

Selected species of barley (*Hordeum vulgare*, *Hordeum spontaneum*, *Hordeum jubatum*) and some wildy growing forms of goatgrass (*Aegilops* sp.) rank among plants tolerant to salinity. As opposed to that, durum wheat (*Triticum durum*) is known for its low tolerance to salinity. It depends,

not only with barley but also with other plants, on what development phase the given plant is in when exposed to the salinity conditions (Munns, 2002). Mano and Takeda (1997) cite the germination period as the critical development phase. Pandya et al. (2005) confirmed that barley is tolerant to salinity exactly during the germination period.

It was found out that the genotypes tolerant to salinity have the capability to expel the undesirable ions. Garthwaite et al. (2005) identified this ability in the wild-growing barleys, which have higher tolerance to salinity as compared to other cultural types. Fricke and Peters (2002) observed that the elongation growth in the hydroponic cultures of spring barley was slowed down at the third-leaf stage due to the decrease of the water and osmotic potentials. The plants in a salinated environment are not only exposed to the effects of salt ions but they are also exposed to the low osmotic potential of the soil solution. It generally applies that if the rate of decrease of water potential exceeds 1.1 MPa per day, the plant is unable to adapt at a sufficient speed (Kramer, 1983).

Based on the facts mentioned above, we presume that the various genotypes of barley can demonstrate not only various levels of tolerance to salinity but also various physiological adaptations to this stress factor. In order to verify this hypothesis, we chose six different genotypes of spring barley and we examined their physiological response to salinity by

monitoring the growth of leaves and roots, the osmotic and water potentials of leaves, the photosynthesis and transpiration rates and the entry of ions Na^+ into a plant. The goal was to find out how the individual varieties adapt to the induced stress conditions, for example, whether they reduce growth or whether they reduce water or osmotic potential or whether they perhaps do both at the same time. The obtained results could be used in breeding to obtain more resistant plants.

MATERIALS AND METHODS

Six varieties of spring barley were selected as the experimental material (*Hordeum vulgare* L.): *Amulet* (a Czech variety), *Jersey* (a Dutch variety), *Krona* (a German variety), *Malz* (a Czech variety), *Norimberk* (an older German variety), *Valticky* (an older Moravian variety). During the previous experiments, differences between the varieties in their water management had already been identified.

The cereal grains were pre-germinated in an air-conditioned chamber at the temperature of 20 °C. Three days later, the young seedlings were replanted into containers with Knop's nutrient solution (nutrient concentration 21.26 mmol l^{-1} : K^+ 3.4, Ca^{2+} 3.4, Mg^{2+} 0.81, Fe^{3+} 0.66, NO_3^- 8.84, H_2PO_4^- 3.34, and SO_4^{2-} 0.81 mmol l^{-1} , pH 5.73) and thereafter they were grown as a hydroponic culture. During the cultivation, stable photon flux density of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was set in the air-conditioned chamber; the duration of the day was set at 14 hours; the temperature was set at 22 °C during the day / 18 °C at night; and the air humidity was between 40 and 60 %. Before the third leaf started to be formed (stage 12th DC), the plants were divided into 4 groups: in the first group, the control, non-stressed plants were cultivated in Knop's nutrient solution; in the other three groups, stress conditions were induced for the plants by adding 0.06M, 0.15M and 0.25M solution of osmotically active substance NaCl into the Knop's nutrient solution. The plants were being stressed under the salinity conditions for five days.

The gas exchange parameters (photosynthesis rate Pn and transpiration rate E) were measured gasometrically in intact plants at the phase of the third fully developed leaf (13th DC)

by means of the LCpro+ apparatus (*ADC Bio Scientific Ltd.*). The measurements were carried out in an air-conditioned chamber; the temperature in the measuring leaf chamber was 25 °C; photon flux density was 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$; the air humidity in the leaf chamber was 50 %; and the concentration of CO_2 in the air incoming to the leaf chamber was 350 $\mu\text{mol mol}^{-1}$. The taken and frozen samples of leaves were used, after defrosting, to identify psychrometrically the osmotic potential from the cell sap (Smith et al., 1992). The water potential of the plants was identified psychrometrically on leaf disks by means of the PSYPRO apparatus (*Wescor*). In the phase of the third fully developed leaf, the length of the leaves and the length of the roots were also measured in the plants. In order to assess the impact of Na^+ on the metabolism of the plant, analyses of the plants grown in the control group and under stress conditions induced by 0.15M solution of NaCl were carried out. For the purposes of the analysis, the plants were being dried at 105 °C for a period of 1 hour and were homogenised on a ball mill. The contents of Na^+ and K^+ ions were identified by a laboratory for soil and plant analysis in the Crop Research Institute in Prague by means of an optical emission spectrometer with induction-bound plasma ICP-OES Thermo Jarell Ash (Trace Scan).

The hydroponic cultivation in the air-conditioned chamber was repeated 6 times; each repetition involved 24 containers (6 varieties x 4 versions of the solution; there were 10 plants in each container). Each measurement was carried out for two plants from each container.

The results were statistically examined by the analysis of variance (ANOVA) for significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

With the selected varieties of spring barley, attention was focused on the physiological responses of plants to stress conditions induced by salinity.

It follows from the results shown in Figure 1 that all the examined varieties responded to the graded "salinity" by gradually decreasing the levels of the osmotic potential of the 3rd leaf. They all demonstrated the capability of osmotic adaptation.

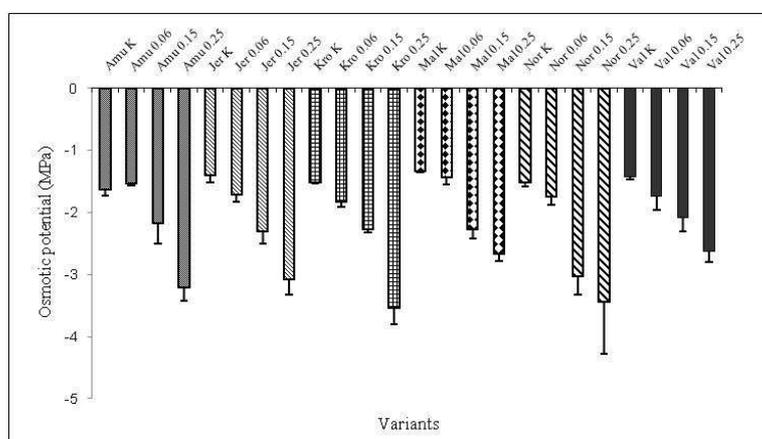


Figure 1. The osmotic potential measured in the 3rd leaves of spring barley plants. Mean \pm SD (n=12). K: control variant (Knop's nutrient solution), Amu: Amulet, Jer: Jersey, Kro: Krona, Mal: Malz, Nor: Norimberk, Val: Valtick

The osmotic adaptation capacity of most mesophyte plants is relatively low and hovers around 1 MPa, depending on the species and the physiological state of the plant. The genetic variability within a plant species is low, for example, around 0.4 MPa for maize. The varieties with a higher capability to accumulate osmotically active substances, which therefore have a capability to reduce the osmotic potential level, can be regarded as plants more resistant to water stress. Under the induced stress conditions with NaCl concentration level of 0.15M, the Norimberk, Malz and Jersey varieties reduced the osmotic potential the most in comparison with the control group; conversely, the Amulet variety demonstrated the smallest reduction of the osmotic potential. At the 0.25M concentration, the highest osmotic potentials were identified in the Valticky and Malz varieties. A medium decrease of the osmotic potential was identified in the Jersey and Amulet varieties and the lowest osmotic potential was observed in the Krona and Norimberk varieties. At the 0.25M concentration, the osmotic potential decreased by highly significant numbers when probably uncontrolled intrusion of NaCl into the tissues was already taking place. This was

manifested by stagnation of growth, visible damage and by the tissues turning yellow. This is why the 0.15M concentration was thereafter used to examine the impact of salinity on selected physiological parameters.

The said results are in accordance with the results published by Reggiani et al. (1995), who proved differences in osmolarity in various wheat varieties in connection with increasing external concentration of salt.

It follows from the results shown in Figure 2 that all the varieties demonstrated the capability to decrease the water potential of leaves and hence the capability to adapt to salinity induced by adding 0.15M NaCl to the nutrient solution. In comparison with the control group, the Amulet variety decreased the water potential the most; the smallest decrease of the water potential was observed, based on the measurement, in the Valticky variety. However, it can be assumed that the data obtained from the measurements do not indicate the maximum capability of the individual varieties to reduce the water potential level.

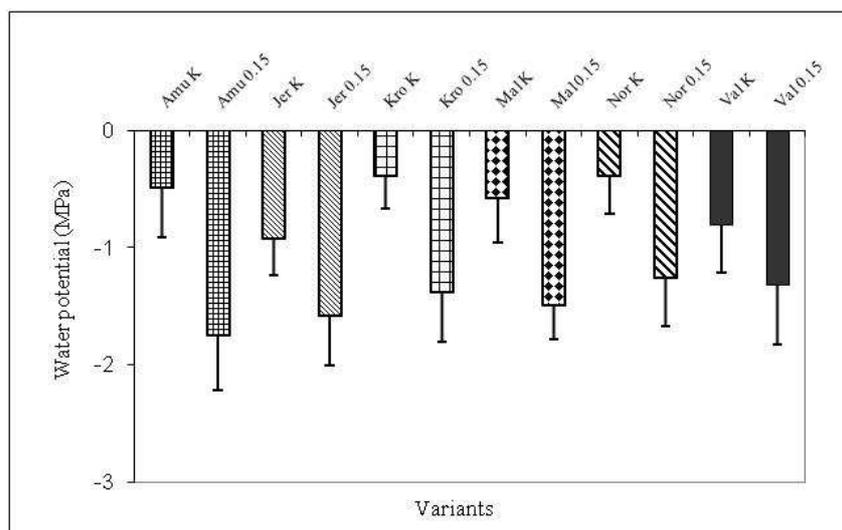


Figure 2. The water potential measured in the 3rd leaves of spring barley plants. Mean \pm SD (n=12).

In order to limit the losses in agricultural crop production, it is very important to get to know the physiological adaptation mechanisms, which the individual genotypes of plants use to control the parameters of gas exchange under reduced availability of water (Hoffmann and Burucs, 2005; Balla et al., 2006).

It follows from the results shown in Table 1 that with respect to transpiration rate, the transpiration rates identified in all the varieties during the measurements decreased in correlation to the increase of the concentration of salt in the nutrient solution. The 0.25M concentration of NaCl was harmful to all the varieties – there was a significant decrease of the gas exchange parameters, that is, a significant decrease of the photosynthesis and transpiration rates. With respect to photosynthesis rate, the Norimberk and Jersey varieties

responded most sensitively and therefore the greatest reduction of the photosynthesis rate occurred in these varieties. The response to lower salt concentration levels was variety-specific. At the 0.15M concentration of NaCl, the Amulet and Valticky varieties demonstrated a photosynthesis rate statistically comparable with the photosynthesis rate under the control non-stress conditions. In the other varieties, a statistically significant decrease of the photosynthesis rate levels was identified in the measurements. It can be assumed that under a more slowly appearing and longer-term stress in field conditions, maintaining a higher rate of pure photosynthesis can be an important mechanism to achieve higher yields (Brestic et al., 2007). Safrankova et al. (2007) or Degl'innocenti et al. (2009) also achieved differences at the level of genotypes and different groups.

Table 1. The photosynthesis rates and the transpiration rates measured in the 3rd leaves of spring barley plants. Mean \pm SD (n=12).

Characteristic	Varieties	Variants			
		K	0.06M	0.15M	0.25M
Rate of photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Amulet	13.82 \pm 1.68 a	14.62 \pm 0.63 a	12.86 \pm 1.21 a	7.06 \pm 1.05 b
	Jersey	15.06 \pm 0.52 a	13.27 \pm 3.67 a	4.58 \pm 1.70 b	2.31 \pm 0.22 c
	Krona	13.47 \pm 2.03 a	17.87 \pm 0.70 b	9.86 \pm 1.20 c	7.90 \pm 1.47 c
	Malz	12.59 \pm 0.20 a	9.67 \pm 0.81 b	5.98 \pm 1.39 c	5.92 \pm 0.85 c
	Norimberk	12.89 \pm 0.27 a	7.93 \pm 0.66 b	5.29 \pm 0.25 c	1.71 \pm 0.97 d
	Valticky	13.26 \pm 1.28 a	11.36 \pm 1.90 a	11.77 \pm 1.37 a	5.30 \pm 3.54 b
Rate of transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	Amulet	2.94 \pm 0.15 a	2.59 \pm 0.47 a	1.88 \pm 0.25 b	0.37 \pm 0.08 c
	Jersey	2.87 \pm 0.36 a	2.89 \pm 0.36 a	1.18 \pm 0.60 b	0.69 \pm 0.13 c
	Krona	2.84 \pm 0.13 a	2.88 \pm 0.90 a	2.00 \pm 0.81 b	1.06 \pm 0.58 c
	Malz	2.77 \pm 0.13 a	1.96 \pm 0.23 b	0.91 \pm 0.50 c	0.58 \pm 0.55 d
	Norimberk	2.87 \pm 0.20 a	1.81 \pm 0.56 b	1.17 \pm 0.64 c	0.68 \pm 0.26 d
	Valticky	2.69 \pm 0.37 a	2.93 \pm 0.43 a	1.58 \pm 0.81 b	1.15 \pm 0.27 c

The ratio of photosynthesis rate and transpiration rate, referred to as water use efficiency (WUE = Pn/E), can show the water management capabilities of the individual varieties as well as their capabilities to photosynthesise and produce under stress conditions. With regard to this capability, we can divide the examined varieties of spring barley into two basic groups:

1. The varieties with a provable increase of WUE, that is, with the capability to manage water better under stress conditions (Amulet, Malz, Krona).
2. The varieties with stagnation or decrease of WUE levels, which are consequently more sensitive to salinity (Valticky, Norimberk and Jersey).

Table 2 shows the results of the analysis of the plants' leaves with a focus on the Na⁺ and K⁺ ion content. The plants grown in the control group (Knop's nutrient solution) and in the group exposed to salinity with the 0.15M concentration of NaCl were assessed. NaCl in the nutrient solution has a crucial influence on the intake of Na⁺ ions into leaves. The Valticky variety accumulated the highest quantity of Na⁺; the Krona variety accumulated the lowest quantity of Na⁺. At the same time, the potassium ion (K⁺) content levels in the leaves decreased in all the varieties. The greatest decrease of K⁺ content occurred in the Krona variety, which was followed by Norimberk, Jersey, Amulet and Malz. The smallest decrease of the K⁺ content in the leaves occurred in the Valticky variety. Thus, differences between varieties were identified.

In a similar experiment with spring barley, Widodo et al. (2009) also observed differences between varieties. Saffan (2008) was also trying to ascertain the content of inorganic substances Na⁺, Cl⁻ and K⁺ in the dry matter of the leaves of barley under stress conditions induced by salinity with 0.2M concentration and he found out that the Na⁺ content increased significantly and that the K⁺ content decreased. In the paper by Khosravinejad et al. (2009b), it was found out that as the concentration of salt in the nutrient environment increases, the concentration of Na⁺ in the cereal grains of barley increases, while the concentration of K⁺ decreases.

Table 2. The K⁺ and Na⁺ ion contents measured in the leaves of spring barley plants.

Varieties	K ⁺ in dry matter of the leaves (mg kg ⁻¹)		Na ⁺ in dry matter of the leaves (mg kg ⁻¹)	
	K	0.15M	K	0.15M
Amulet	84648	61861	319	18278
Jersey	94198	65323	393	19050
Krona	101467	63263	521	13787
Malz	84094	69294	507	16260
Norimberk	87143	55222	467	17852
Valticky	81467	80041	513	23058

Begum et al. (2008) examined a similar correlation between Na⁺ and Cl⁻ on one hand and K⁺ on the other hand in wheat and they found out that salinity induces reduction of growth caused by accumulation of excessive amounts of Na⁺ and Cl⁻ with the simultaneous decrease of the K⁺ content. Tuna et al. (2008) examined the effect of the accumulation of Na⁺ in the leaves and in the roots of durum wheat (*Triticum durum*) and common wheat (*Triticum aestivum*). Based on their research, they arrived at the conclusion that common wheat is more tolerant to salinity than durum wheat. They justify this conclusion by stating that the accumulation of Na⁺ in the roots indicates a positive mechanism, by means of which common wheat copes with the salinity in the soil, or that it may indicate the existence of a mechanism inhibiting transport of Na⁺ into leaves. Based on this fact, it is therefore possible that a similar mechanism also works in spring barley.

The growth of leaves, which is assessed in Table 3, is highly sensitive to low levels of water potential and turgor. Already a small decrease of water potential and turgor can result in a significant reduction and possibly even termination of the growth of leaves. The fact that high concentration of salt is toxic for a plant and limits its growth has been confirmed for barley, for example, by Endris and Mohammed (2007). Khosravinejad et al. (2009a) explain that the harmful effect of salinity on the growth of plants is the result of the direct influence of toxic ions or the indirect

influence of salt on soil water potential, resulting in an osmotic imbalance in the relation between the soil and the plant.

As the results in Table 3 show, the modern varieties Amulet, Jersey, Krona and Malz respond to slight salinity (0.06M NaCl) with a slight increase of growth in comparison with the control group. Conversely, the older varieties Norimberk and Valticky are sensitive and their growth decreased already at this salt concentration. At higher NaCl concentration levels (0.15M and 0.25M), the growth of

leaves of the examined varieties declined. The Amulet and Malz varieties had the most sensitive response and the shortest leaves were observed in these varieties; and the same was true for the Norimberk variety with the 0.25M concentration level. The fact that if plants are exposed to salinity under laboratory conditions, a fast and temporary decrease of their growth will occur, was also confirmed, for example, by Begum et al. (2008) who studied the effect of salinity on wheat.

Table 3. The length of the 3rd leaves of spring barley plants. Mean \pm SD (n=12).

Varieties	K	The length of the 3 rd leaves (mm leaf ⁻¹)		
		0.06M	0.15M	0.25M
Amulet	112.60 \pm 17.70	114.20 \pm 27.40	62.40 \pm 32.20	56.60 \pm 18.80
Jersey	96.60 \pm 19.40	159.00 \pm 02.30	110.4 \pm 27.00	82.00 \pm 19.10
Krona	110.20 \pm 7.90	137.80 \pm 17.30	105.20 \pm 39.00	74.40 \pm 9.10
Malz	93.20 \pm 37.30	105.40 \pm 25.80	46.20 \pm 14.30	32.60 \pm 23.50
Norimberk	145.40 \pm 14.40	119.20 \pm 31.60	105.00 \pm 22.40	30.60 \pm 9.00
Valticky	120.20 \pm 31.10	77.20 \pm 20.30	95.00 \pm 13.40	68.40 \pm 21.30

The properties of the root system are also important for assessing the varieties with respect to their tolerance to salinity or drought. For example, the length and diameter of a root and the volume of the root hairs are important. As the results in Table 4 show, the growth of roots in all the examined varieties is reduced under the stress conditions of salinity at the 0.15M concentration of NaCl in the nutrient solution. Similarly like in the experiment carried out by Khosravinejad et al. (2009a), the roots in the salinated group were shorter and often had lower number of short branching parts in comparison with the control group grown in Knop's nutrient solution. Strong reduction effects of salt on the prolongation growth of roots were also confirmed by Moud and Maghsoudi (2008) for wheat. In their experiment, a stronger salinity-related reduction was observed in the above-ground part in comparison with roots. They drew the conclusion from the results that the length of roots can be used as the selection criterion for tolerance to salinity in early growth phases. They also hold the opinion that such varieties, which are capable of prolonging growth of both roots and the above-ground part under salinity conditions, should be used for breeding.

Table 4. The length of the roots of spring barley plants. Means \pm SD (n=12).

Varieties	The length of the roots (mm root ⁻¹)	
	K	0.15M
Amulet	121.00 \pm 16.36	81.60 \pm 10.97
Jersey	168.40 \pm 17.95	84.80 \pm 17.18
Krona	139.00 \pm 4.64	65.60 \pm 10.33
Malz	113.60 \pm 2.97	90.20 \pm 12.26
Norimberk	125.00 \pm 18.59	83.20 \pm 21.00
Valticky	135.00 \pm 3.54	79.40 \pm 5.03

It follows from Table 4 that the greatest reduction of root growth was observed in the Jersey variety, whose roots

exposed to salinity were shorter by 83.6 mm in comparison with the control group, and in the Krona variety where the difference was 73.4 mm. Conversely, the smallest difference between the control group and the salinated group, specifically 23.4 mm, was observed in the Malz variety.

If there is a long-term lack of water since the beginning of the vegetation period, first roots prolong in order to reach greater depths but this takes place at the expense of the creation of lateral roots and root hairs. If the water stress continues, the root system is reduced; it stops creating root hairs and, eventually, complete reduction of the growth of roots occurs and the root system dies. The response of roots, that is, their capability of a morphological change after the onset of drought, is often deemed to be one of the most important factors of resilience.

Based on the comparison of the lengths of leaves and roots, it can be said that after 5-day exposure to 0.15M concentration of NaCl, the Amulet and Malz varieties reduced the growth of leaves more than the growth of roots as compared with the control non-stress group. The other varieties responded in an opposite way. It is exactly the changes in the growth rate of leaves and roots in individual varieties under decreased water availability that can be an important selection criterion concerning tolerance to drought and salinity.

In conclusion, it can be stated that the assessed varieties of spring barley responded to salinity stress in various ways – by reducing the osmotic and water potential, by reducing gas exchange parameters and by changing water use efficiency, by accumulating Na⁺ ions and by reducing the growth rate of leaves and roots. And yet individual varieties demonstrated different levels of sensitivity of the assessed physiological characteristics to different levels of salinity. After the 0.15M solution of the osmotically active substance NaCl was added into the nutrient solution and after 5-day exposure, the highest capability of osmotic adaptation, that is, the greatest

reduction of osmotic potential was observed in the Norimberk, Malz and Jersey varieties. The decrease of the osmotic potential of the Amulet variety was the lowest. After the use of the 0.15M concentration of NaCl, the photosynthesis rate in the Amulet and Valticky varieties was comparable with the photosynthesis rate under non-stress conditions. Conversely, the greatest reduction of the photosynthesis rate, after the use of higher concentration levels of the salt, occurred in the Norimberk and Jersey varieties. With respect to water use efficiency, the Amulet, Malz and Krona varieties demonstrated better water management under the salinity conditions than the Valticky, Norimberk and Jersey varieties did. When the 0.15M concentration of NaCl was used for cultivation of the barleys, the Valticky variety accumulated the highest quantity of Na⁺ ions in its leaves and the Krona variety accumulated the lowest quantity of these ions. At the same time, the content of K⁺ ions in the leaves decreased in all the varieties. The greatest decrease in the content of K⁺ occurred in the Krona variety and the smallest decrease occurred in the Valticky variety. Also, depression in the growth of leaves occurred in all the examined varieties. The Amulet and Malz varieties demonstrated the most sensitive response and based on measurements, these varieties were found to have the shortest leaves. The growth of roots was reduced in the Jersey and Krona varieties the most, while in the Malz variety, it was reduced the least. In the Amulet and Malz varieties, greater reduction of the growth of leaves than the growth of roots was observed in comparison with the control non-stress group after 5-day exposure to 0.15M concentration of NaCl. In the other varieties, the response was the opposite.

The physiological characteristics assessed (water and osmotic potential, photosynthesis rate, transpiration rate, water use efficiency, the Na⁺ and K⁺ ion content in leaves and the growth of leaves and roots) indicate the existence of complicated interactive relations in the adaptation of plants to salinity. This is why future research into the effect of the harmful ions of salt on the adaptation of plants in arid and semi-arid regions is justified and promising.

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