DIFFERENCES IN PROLINE CONTENT AND OSMOTIC POTENTIAL IN SPRING BARLEY VARIETIES GROWN UNDER SALINE CONDITIONS*

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In the experiment the effect of salination in six varieties of spring barley was monitored. Both modern varieties (Jersey, Malz, Krone and Amulet) and older varieties (Valticky and Norimberk) were used for the experiments. The plants were cultivated under controlled conditions in an air conditioned chamber using hydroponics. The older variety Norimberk showed the lowest osmotic potential under the salinity conditions, whereas in modern varieties Malz and Krone the highest osmotic potential was found. An increase in proline content in the treated variants compared to the controls was recorded for all monitored spring barley variants under the salination conditions. The highest proline content was shown for older varieties Norimberk and Valticky as well as the modern variety Jersey. A positive correlation between the proline content and osmotic potential in the leaves of spring barley were cultivated under the conditions of salination was observed.

cereal; nutritive solution; salination; osmotic potential; refractive index

INTRODUCTION

The salination of cultivated agricultural areas is a global problem, particularly in semi-arid and maritime areas (Munns, 2002). The main reason for studying plant resistance to salination is the fact that more than one third of the Earth's inhabitants must resolve the issue of soil salination. Therefore the screening and assessment of the physiological response of salinity tolerant genotypes (El – Hendawy et al., 2007) is carried out. The most sensitive plants subsequently respond by growth decrease and changes in metabolic processes (Reggiani et al., 1995).

Salination causes the soil water potential to decrease on the one hand, which is simultaneously the cause of drought stress as well as salt ion toxicity, these ions are taken up from the soil and accumulate in plant cells and thus affect the imbalance in plant nutrition. A decrease of the leaf tissues' water potential occurs, eventually leading to the accumulation of osmotically active substances, for example carbohydrates or amino acids, proline, in particular. The effect of 150 mmol NaCl on three wheat varieties was monitored, where a negative influence of chloride ion toxicity as a result of a change in the pH of the environment was found (Reggiani et al., 1995). The osmolarity grew more for the shoots, inferred solutions involved in osmoregulation were carbohydrates, proline and glycine betaine. These compounds are closely related to the osmotic adjustment, protection and function of the cell structure, they are referred to as energy supply substances. It was also proven that sorghum plants exposed to stress accumulate these osmotically active substances (de Lacerda et al., 2003, 2005).

From the point of view of tolerance we can distinguish three basic groups of genotypes, sensitive to salination, tolerant and able to avoid stress. Proline accumulation is a long-term known response to salination. But different authors disagree, if proline formation is directly related to salination tolerance (Poustini et al., 2007). Generally, barley is considered as a crop with a high salination tolerance. Jiang et al. (2006) shows that, as a field crop, barley is ranked among the most tolerant crops, however, between particular genotypes and lines differences were found. The salinity susceptibility index (SSI) is used to define differences between genotypes. Garthwaite et al. (2005) investigated the properties of eight wild barley genotypes to establish osmotic potential and the content of selected amino acids and their amides. In comparison with *Hordeum vulgare* and *Triticeae*, wild genotypes showed a higher salination tolerance. For sensitive varieties the accumulation of osmotically active substances occurs, which enables osmotic adjustment (Maggio, 2002). Carbohydrates are primarily ranked among these active substances, but there are also, for

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example, certain amino acids, such as proline. Proline belongs to the substances, through which the plant can overcome the stress load. The primary path of proline synthesis results from glutamic acid. The first reaction of this synthesis is catalysed by glutamate kinase enzyme (EC 2.7.2.11). Proline synthesis is referred to as the initial stage of hydroxyproline formation, which creates a significant element of the plant cell wall, this being as a compound of glycoproteins. The proline content in plants increases independently of the cause of stress (Rhodes et al., 1999). The proline content is also related to photosynthesis, because through its degradation δ-aminolevulinic acid synthesis occurs in an organism, which is the precursor for the porphyrin skeleton of chlorophyll.

Within the context of explaining plant defence mechanisms the author of trials describes the dependence of proline accumulation in plants on the external environment. The response of barley seedlings, subjected to salt stress conditions under different light regimes before UV-B radiation was investigated. Salt treatment resulted in a decrease of total chlorophyll and free proline contents. Significantly more proline was accumulated in the light than in darkness (Fedina, 2005, 2006). Foliar soluble proline levels for *Hordeum vulgare* were examined also for *Glycine max* seedlings, which had been fumigated with SO_2 and harvested each under a variety of conditions. The soluble proline levels of necrotic and non-necrotic areas of fumigated *Hordeum vulgare* leaves were greater than those of comparable areas of non-fumigated leaves. Proline accumulation was screened in the plants cultivated under the presence of metal ions. In the leaves of rice grown under an increased concentration of cuprum ions (Chen et al., 2001), an increase of proline content was observed as well as in the above-ground and root parts of barley plants cultivated under the presence of nickel ions (Gajewska, 2006). An increased proline level was observed in various plant parts of the plants grown under the salination conditions (Jiang et al., 2006).

MATERIAL AND METHODS

Varieties with inferred varying capabilities of salinity tolerance were chosen from previous experiments. The modern varieties (Jersey, Malz, Krone and Amulet) and older varieties (Valticky and Norimberk) were used for the experiment. Seeds were obtained from the Agricultural Research Institute Kroměříž, Ltd. The plants were grown under controlled conditions in an air conditioned chamber, where the following parameters were set. The length of the lighting period was 14/10 hours, temperature 22/20°C and moisture 40–60%. The plants were cultivated under a lighting of 300 µmol m^{-2} s^{-1}. The plants were germinated in a thermostat at a temperature of 20°C, when the coleoptile size was 150 mm they were replanted in modified boards for hydroponics cultivation fixed with foam rubber and with 10 plants in cultivation pots with a volume of 0.5 l in Knop's nutritive solution. Three repetitions were made for each cultivated varieties, nutritive solutions were regularly refilled by water. After three weeks of cultivation stress through salination was induced by adding NaCl at a concentration of 0.15 mol, to the nutritive solution, which corresponds with an osmotic potential value of –0.7 MPa. The concentration was selected from previous experiments, where this concentration showed changes in the plants physiological processes, compared to controls, but did not stop them. The plants endured salinity stress for five days and then the entire plants were sampled and lyophilised. The lyophilised plants were ground into a fine powder, samples were homogenised and stored in the refrigerator and prepared for further analyses. The lyophilised plant material meant for determining the proline content was homogenised with sea sand and the homogenate was centrifuged for a period of 20 min under 10,000 revolutions per min. The supernatant was derivatised and subsequently analysed according to the standard methodology for amino acid ACCQ analysis by means of the HPLC method for reverse phase with fluorescence detection (WATERS, Milford, USA). Amino acid were analysed and the records assessed using chromatographic software EXPLORER. Part of the samples was taken for measuring the osmotic potential. The entire plants were inserted into syringes, frozen and, after thawing, the sap was squeezed from the leaves. All measurements were repeated five times by means of the psychrometrical device PSYPRO and HR33 in chambers C-52 (WESCOR Inc. An Elitech Company, USA). Identical samples of cell sap were measured using the ABBE refractometer and the substances' refractive indexes were determined.

The salinity susceptibility index SSI (%) = $(Xs/Xc) \times 100$ was used to define the differences between particular varieties, where Xs is the average value of the salinised variant, Xc is the average value of the control variant.

The effect of salinity and differences in osmotic potential, refractive index and proline content were analysed using an ANOVA test. The significance of differences between the means was assessed using the Tukey test and LSD test. The coincidence coefficient was set at 0.05.

RESULTS AND DISCUSSION

The differences in the osmotic potential values were measured between the non-stressed as strong stressed variants (Fig. 1). From the osmotic potential measurements we determined that for the non-stressed variants (LSD-test) the variety Norimberk differed with a higher osmotic potential in comparison to other

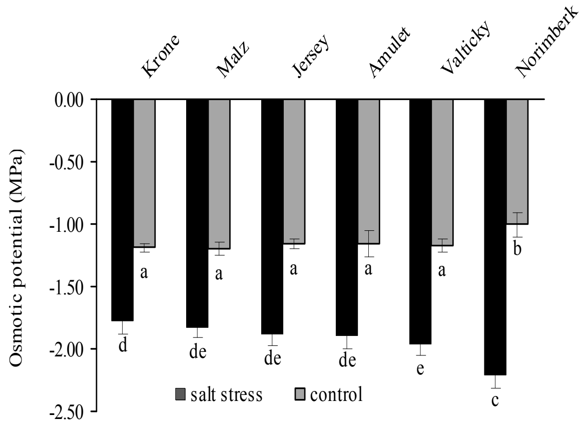

Fig. 1. Osmotic potential for experimental (salt stress) and control variants of barley varieties in the stage of 3rd true leaf fully expanded, measured from the squeezed sap of the experimental plant leaves after a five-day application of 0.15M NaCl added to the nutrient solution

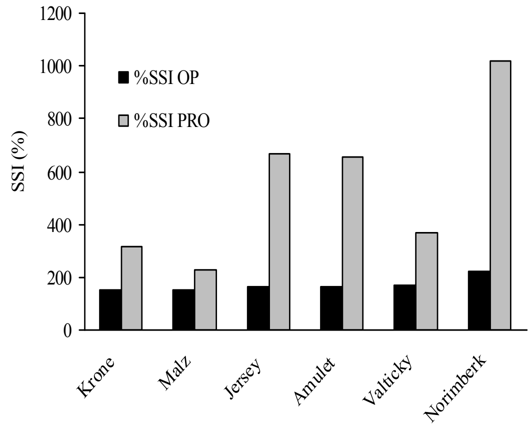

Fig. 2. Salinity susceptibility index SSI % calculated for the change in osmotic potential (OP) and change in proline content (PRO) for barley varieties in the stage of 3rd true leaf fully expanded, measured of the experimental plant leaves after a five-day application of 0.15M NaCl added to the nutrient solution

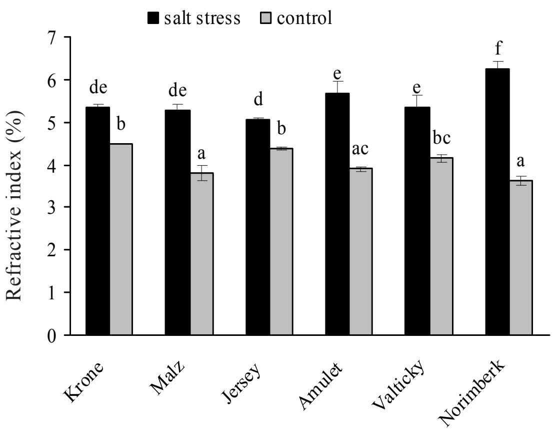

Fig. 3 Changes in refraction index in experimental (salt stress) and control variants for barley varieties in the stage of 3rd true leaf fully expanded, measured from the squeezed sap of the experimental plant leaves after a five-day application of 0.15M NaCl added to the nutrient solution

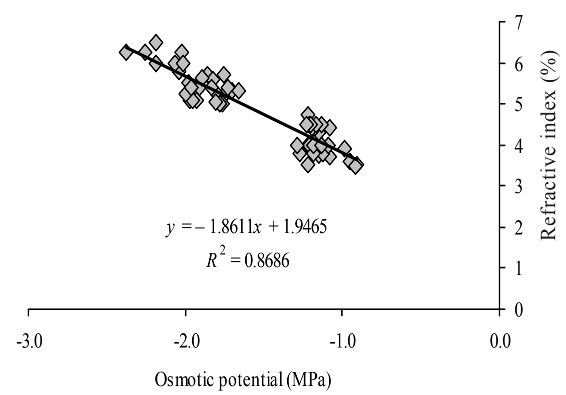

Fig. 4 Correlations of the refraction index's dependence on osmotic potential determined from identical samples of sap squeezed from barley leaves of control and experimental variants

varieties. For the stressed variants osmotic potential decreased in all varieties and when using the LSD test, then the varieties Norimberk from Valticky, Amulet and Jersey from Malz and Krone differ. The measurements were assessed by analysis of variance at a significance level 0.05.

The percentage increase of the potential in the experimental variant compared to the control plants has been calculated as a salinity susceptibility index SSI(%) for the change in osmotic potential for the varieties, where the lowest changes, 150%, have been measured for the variety Krone and Malz, slightly higher, 160%, for Jersey, Amulet and Valticky and distinctively higher, 250%, for the variety Norimberk (Fig. 2).

By means of the refractometric determination of the cell sap's refraction index it was again found, using the Tukey test, $\alpha = 0.05$ that there are differences both between the control variants and within the salt stressed cultivars. For the control variants we determined differences of lower values for the varieties Norimberk, Malz, Amulet and higher values for varieties Valticky, Jersey, Krone (Fig. 3). For the salt stressed cultivars higher indexes of refraction for all varieties were found in comparison with the non-stressed variant, the variety Jersey with the lowest value and the variety Norimberk with the highest value of the refraction index differed. Correlations of the refraction index's dependence on osmotic potential determined from identical samples of sap squeezed from barley leaves of control and experimental variants was observed (Fig. 4).

The samples of six barley varieties have been analysed for proline content in the control as well as experimental variants. For the analysis mixed samples were prepared and the proline content was defined as the mean from two repetitions of the measurement in μmol/mg of the dry weight of the analysed plant samples, where the RSD obtained values were within

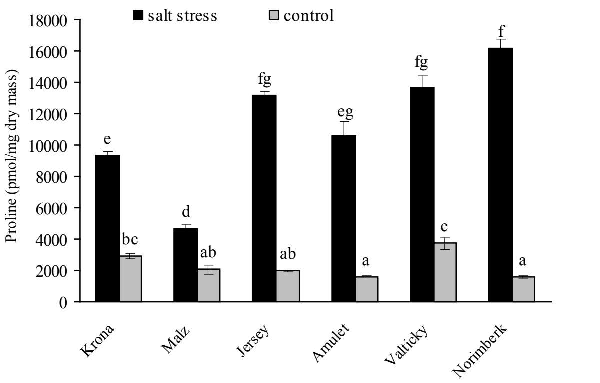

Fig. 5. Proline content in experimental (salt stress) and control variants for barley varieties in the stage of 3rd true leaf fully expanded, measured from homogenised samples of the experimental plant's leaves after a five-day application of 0.15M NaCl added to the nutrient solution

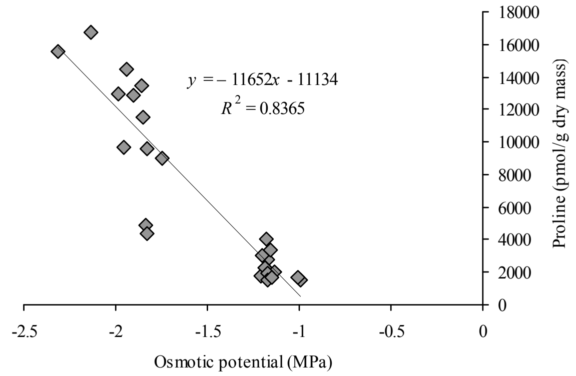

Fig. 6. Correlation of proline content dependence on osmotic potential measured from the spring barley leaves and control and experimental variants after a five-day application of 0.15M NaCl added to the nutrient solution in the stage of 3rd true leaf fully expanded

the interval of 1–11. As it is evident from Fig. 5 for all screened varieties an increase in proline content was recorded for the experimental variants compared to the control. But for particular varieties the proline content was distinct both for experimental variants and control variants. The highest content was found in the salt stress variant for the varieties Norimberk (16 µmol/mg), Valticky and Jersey. For the varieties Amulet, Krone and Malz was determined the lowest proline content.

For comparing the varieties from the point of view of a different proline content synthesised due to salination a statistical evaluation of the proline contents was used for all six varieties. The results of Tukey's HSD test at the level $\alpha = 0.05$ gave evidence for a certain similar protective mechanism in barley varieties enabling adaptation to a salinity stress load. From the graphical depiction (Fig. 5) a significant differences in proline accumulation between the varieties Norimberk and Malz were found. The statistical evaluation demonstrated that the varieties significantly differ from one another in proline accumulation. The proline contents indicate that particular barley varieties have different capabilities to synthesise this amino acid as a result of a salinity stress load effect.

To compare proline content in the control and experimental variants for particular varieties, the SSI(%) (Fig. 2) was determined. Significant growth (1019%) in the experimental variant in comparison with the control variant was recorded for the variety Norimberk, further for the varieties Amulet and Jersey the proline content increased up to 670% and 558%, for Valticky (369%) and Krone (319%) and the minimum growth in proline content for an experimental variant was observed for the variety Malz (227%).

During the screening of SSI values of the osmotic potential of the leaves and proline synthesis it was shown that the level of changes for proline is up to 1000%, whereas for WP 250% at most and the refraction index had a maximum of 80%. Nevertheless, the correlation is positive. With decreasing osmotic potential the proline content increases as do the substances' refraction indexes. The representation of leaf water state through osmotic potential and the refraction index correlates with the proline content.

A correlation of proline content values and osmotic potential for all screened barley varieties was observed. From the dependence presented in Fig. 6 it can be seen that with decreasing osmotic potential the proline content increases. From the regression analysis it can be seen that the given dependence for the monitored cereal is relatively close.

All barley varieties were grown under the defined salinity conditions of a 0.15 mol NaCl solution. The total change in the osmotic potential in the leaves includes not only the NaCl taken up but even possible osmoprotective or detoxication substances (carbohydrates and proline, etc.) that have been formed.

Similarly as was shown in our work also Garthwaite et al. (2005) measured the changes in osmotic potential of cell sap in wild *Hordeum* species under varying concentrations of salt added to the nutrient solution and found changes in the osmotic potentials within the varieties. Under salt stress the variations in osmoregulation were confirmed among three wheat cultivars, these variations are related to the accumulations of ions. The salt accumulation was the main factor determining the variations in osmolarity (Reggiani et al., 1995). Whereas from the measurement of the leaves osmotic potential the water molecules energy was found, the refractometric determination of the refraction index rather assesses the osmotically active substances content. For the measurement of the refraction index higher differences for the control, non-stressed variants were determined in comparison with osmotic potential determination. Despite this the total dependence of the refraction index's values on osmotic potential of the sap from spring barley leaves was obtained.

Proline content was determined in our experimental varieties, similarly as in other works (Bandurska, Stroinski, 2003) where the water state was defined through RWC; correlations between the stress level, to which the plants were exposed, and the amount of accumulated proline were found. Therefore it can be inferred that the ability for higher proline formation may be attributed to wild species *H. spontaneum* rather than to modern barley cultivars. Similarly as in our work the older variety Norimberk shows a higher capability to produce proline as well as a higher level of osmolarity compared to certain modern varieties (Malz, Krone). From the osmoprotective point of view a higher proline level can be important. From the point of view of the metabolism of such significant substances as proteins and carbohydrates the presence of amino acids is probably essential. The proline syntheses of precursors there are changes in the activity of enzymes. For investigating the roles of factors during proline synthesis under salinity stress, the activities of glutamine synthetase (GS; EC 6.3.1.2) and NADH-dependent glutamate dehydrogenase (NADH-GDH; EC 1.4.1.2) were determined in the leaves of wheat (*Triticum aestivum*) seedlings exposed to salt stress. Under the lower salinity conditions, only GS activity increased markedly. However, under a higher salt concentration, NADH-GDH activity increased, while GS activity decreased. A significant accumulation of proline was found only under high-salinity exposure, while glutamate, a proline precursor, increased dramatically under the conditions of low as well as high salinity (Wang et al., 2007).

From our results it is evident that for all screened barley varieties an increase in proline content occurred as a result of increased salt content in the nutrient medium. But it is obvious that for all varieties the same increase in proline content was not demonstrated, which seems to be an effect of particular barley varieties genetic properties, indicating this cereal is able to synthesise the substances, which protect the plant against stress effects (Muramoto et al., 1999). Jiang et al. (2006) also observed differences in the proline content of particular barley varieties cultivated under external salinity conditions.

From the experiments of (Poustini et al., 2007) it can be seen that proline accumulation is more evident in sensitive types of plants, where it plays a less important role in the change in osmotic potential, but performs a protective function for cell membrane and cytoplasmatic enzymes. A high Na^+ ion level is necessary to induce proline synthesis (Kronzucker et al., 2006).

CONCLUSIONS

In this experiment the salinity effect for six spring barley varieties was monitored. To infer different properties modern varieties (Jersey, Malz, Krone and Amulet) and older varieties (Valticky and Norimberk) were used. For defining the leaf water state the values of osmotic potential and the refraction index of the substances were determined. An increase of proline content was recorded for salt stressed variants in comparison with non stressed barley varieties. A positive correlation between the proline content and osmotic potential for barley plants cultivated under the salinity conditions was also observed. The highest proline content was found for the older varieties Norimberk and Valticky as well as for the modern variety Jersey. The results confirmed the hypothesis of a difference in proline content for particular spring barley varieties under conditions of salinity. Under our experimental salinity conditions was found that the older variety Norimberk had the highest proline content and the value of osmotic potential in comparison with other varieties. In the oportunity modern varieties Malz and Krone had the same parameters the lowest.

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Změny v obsahu prolinu a osmotického potenciálu u odrůd jarního ječmene pěstovaných v podmínkách zasolení

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V pokusu byl sledován účinek zasolení u šesti odrůd ječmene jarního. K experimentům byly použity odrůdy moderní (Jersey, Malz, Krone and Amulet) a starší odrůdy (Norimberk a Valtický). Rostliny byly kultivovány v řízených podmínkách klimatizované komory v hydroponické kultuře. Ve stadiu tvorby třetího listu byl přidán NaCl v koncentraci 0,15 mol.l^{-1} a po pěti dnech byly rostliny odebrány k analýze. Po zamrazení vzorků a odtátí byl v buněčné šťávě z listů stanoven osmotický potenciál a index lomu. U všech odrůd se snížil osmotický potenciál u pokusných variant vzhledem ke kontrolním variantám. Byla zjištěna pozitivní korelace hodnot indexu lomu na osmotickém potenciálu šťávy z listů. Z lyofilizovaných vzorků byl stanoven obsah prolinu HPLC metodou s fluorescenční detekcí. Byl zaznamenán nárůst obsahu prolinu v ošetřených variantách oproti kontrolním u všech sledovaných odrůd ječmene pěstovaných v podmínkách zasolení. Nejvyšší obsah prolinu a nejnižší osmotický potenciál byl nalezen u starší odrůdy Norimberk, na rozdíl od moderních odrůd Krona a Malz, kde obsah prolinu byl nejnižší ze všech sledovaných odrůd a osmotický potenciál u odrůdy Krona byl nejvyšší.

obilovina; živný roztok; zasolení; osmotický potenciál; index lomu

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