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ROOT CELERY REACTION ON NaCl AND $CaCl_2$ SALINITY

REAKCJA SELERA KORZENIOWEGO NA ZASOLENIE NaCl i $CaCl_2$

Abstract: The aim of this research was to determine the effect of diverse salinity levels of the soil by NaCl and $CaCl_2$ on the chlorophyll, protein, sugars as well as ascorbic acid content in the root celery (*Apium graveolens* L.var. *rapaceum.* (Mill.) DC) leaves. In the pot experiment the plants were watered with a solution of sodium chloride and calcium chloride containing (100 and 300 mM NaCl) and (50 and 150 mM $CaCl_2$), respectively. The results have shown that contents of tested components in celery leaves to a large extent were dependent upon both the levels of basic salinity and used salt. Basic salinity of both NaCl and $CaCl_2$ caused decreased content of the total chlorophyll, protein and soluble sugars in leaves of celery; the stronger the soil salinity was the greater the change was. Soil salinity to large extend influenced also ascorbic acid level in the leaves of celery. Salinity of the soil by $CaCl_2$ at both doses caused an increase of ascorbic acid level of the celery leaves. The other reaction appeared using NaCl, at lower salinity concentration an increase of ascorbic acid was observed, whilst at higher salinity of the soil a decrease of this vitamin has been noticed.

Keywords: salinity, celery, chlorophyll, protein, sugars, ascorbic acid

Plants are subject to various biotic or abiotic stresses coming from their surrounding natural environment including heavy metals, unutilized wastes such as long degrading polymers as well as salinity [1–3]. Salinity is one of the major abiotic stresses affecting germination [4, 5] and plant growth [6, 8]. Plant growth inhibition, resulting in reduced crop yield, has been reported by many investigators [9–14]. Salinity inhibits plant growth in three principle ways: by ion toxicity (mainly of Na^+ and Cl^-), osmotic stress, and nutritional imbalance [15–17]. All of these cause effects on plant growth at physiological and biochemical levels [17].

An unfortunate consequence of salinity stress in plants is the excessive production of *reactive oxygen species* (ROS), such as superoxide, hydrogen peroxide and hydroxyl

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radical. These ROS can seriously disrupt normal metabolism through oxidative damage of macromolecules such as photosynthetic pigments, proteins, nucleic acids and lipids [6, 18–20]. Plants have evolved specific protective mechanisms, involving antioxidant molecules such as ascorbic acid, glutathione, carotenes, α-tocopherol, and enzymes such as superoxide dismutase, catalase, peroxidase and glutathione reductase [4, 6, 19, 21].

Reactive oxygen species play a role in lipid peroxidation, membranes damage and consequently in leaf senescence [21]. Long-term exposure of plants to salinity leads to ionic stress, which can accelerate premature senescence of adult leaves, and also a reduction in the photosynthetic area available to support continued growth [22, 23]. Leaf senescence is marked by decreases in chlorophyll and protein concentration [11, 21, 24]. A decrease in protein content has often been reported as a response of plants to salinization [9, 10, 16, 22, 24–26]. Various researchers have also reported a decrease in chlorophyll content under salt stress [11, 16, 18, 22, 24, 27, 28]. Salinity has an adverse impact on photosynthetic processes inhibiting photosynthetic production (eg sugar) [22].

Calcium plays a crucial role in many plant physiological processes and is essential for plant growth [29]. High NaCl salinity has been shown to induce calcium deficiencies in different plants [15, 30, 31]. In this way, it is known that external application of calcium can ameliorate the adverse effects of salinity on diverse plants species [9, 11, 15, 30, 32].

In the present study, changes in chlorophyll, sugars, ascorbic acid and protein contents of leaves of root celery grown in NaCl and $CaCl_2$ spiked soils are described.

Experimental procedures

Root celery (*Apium graveolens* L.var. *rapaceum.* (Mill.) DC) Odrzanski variety was used in a pot experiment. Tests were conducted under greenhouse conditions using plastic pots with a capacity of almost 5 dm^3. Background parameters of medium were as follows: pH (KCl) = 6.7, 1.6 % humus, as well as macro element contents: 106 mg N, 125 mg P_2O_5, 155 mg K_2O per 1 kg of soil.

Four celery seedlings in the phase of 3–4 leaves were potted per pot in the second half of May. In the first half of July the soil in the pots was treated with sodium and calcium chloride solutions containing 100 and 300 mM NaCl and 50 and 150 mM $CaCl_2$, respectively. The soil of every pot watered maintaining average humidity to 70 % of water holding capacity

Fresh plant material was taken three times with weekly intervals (14, 28, 42 days after medium salinity). Total chlorophyll and ascorbic acid were determined in fresh plant material [33, 34]. In dry matter of plants contents of total soluble sugars according to the Luff-Schoorl method and total nitrogen (accounted onto protein) according to the Kjeldahl method were determined [34, 35].

Statistical analysis was performed on all the quantitative results. Analysis of variance was performed using the Fischer-test F and Tukey's test. The least significant differences among mean values were calculated at $p < 0.05$ confidence level.

Results and discussion

In the experiments applied soil salinity levels using both sodium chloride and calcium chloride affected total chlorophyll, soluble sugars, ascorbic acid and protein contents of root celery leaves (Tables 1–4, Fig. 1).

Measurements of total chlorophyll levels in leaves during the experiment showed changes in the contents of these pigments (Table 1). Changes were dependent upon both time of analysis and levels of soil salinity. Initially during plant growth total chlorophyll content has been observed to increase and thereafter to decrease. During the whole test period total chlorophyll content per fresh matter of leaves of root celery growing in media with both saline sodium chloride and calcium chloride were maintained at lower levels as compared with background soil. A decrease of total chlorophyll in celery leaves have been observed, related to the strength of the soil salinity (Fig. 1). Taking into account average values of total chlorophyll contents of all analysis periods, for

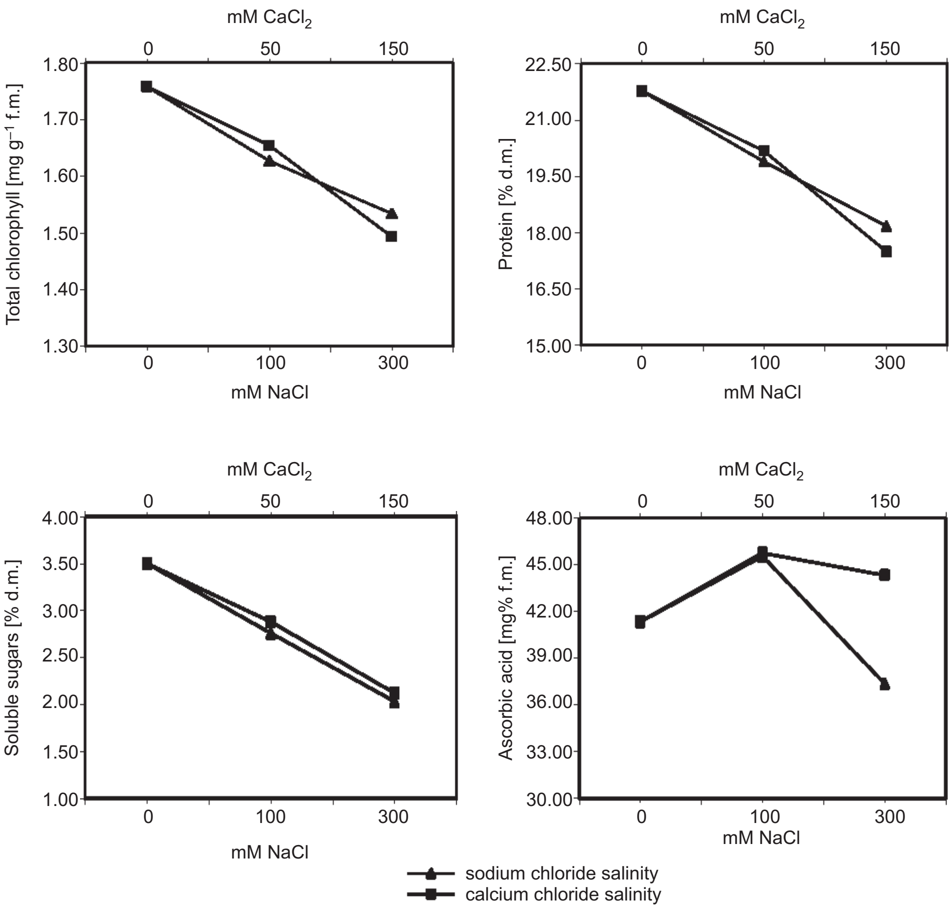

Fig. 1. Changes in nutrition values during exposure plant to salinity. Comparison of both applied salts

higher levels of salinity (300 mM NaCl and 150 mM $CaCl_2$) decreases close to 13 and 15 %, respectively have been noticed as compared with control plants.

Table 1

Changes of total chlorophyll content [mg g^{-1} f.m.] in leaves of root celery during salt treatments

Days	Salinity level of NaCl			Salinity level of $CaCl_2$		
	Background	100 mM	300 mM	Background	50 mM	150 mM
14	1.780	1.671	1.544	1.780	1.645	1.482
28	1.810	1.644	1.588	1.810	1.804	1.525
42	1.683	1.566	1.470	1.683	1.514	1.472
Average value	1.758	1.627	1.534	1.758	1.654	1.493
$LSD_{0.05}$	For time periods – 0.089 For salinity levels – 0.089			For time periods – 0.074 For salinity levels – 0.074		

Among reports concerning reactions of cultivated plants to medium salinity many indicate chlorophyll as an important factor for changes in physiology. The majority of the reports include affects of NaCl on soil, whilst barely a few concern $CaCl_2$ as a factor of salinity. Our results coincide with those obtained by many researches which found that soil salinity activated by NaCl lead to a decrease of total chlorophyll in plants [4, 5, 8, 9, 18, 22, 24, 27, 28, 36–39]. Unexpectedly addition of small amounts of $CaCl_2$ reduce the decrease of total chlorophyll content in leaves of crop plants [11]. Jimenez et al [40] have shown that a mixture of equal amounts of NaCl and $CaCl_2$ leads to a decrease of total chlorophyll content. Our results revealed that high levels of $CaCl_2$ strongly resulted in lowering total chlorophyll of root celery. A decrease of chlorophyll levels induces acceleration of senescence processes of leaves [11, 24].

Moreover, Lutts et al [24] report that salinity causing untimely senescence of leaves besides decreasing chlorophyll levels also induced lowering of protein content. Decrease of protein content is proportional to the extent of medium salinity as a result of NaCl stress [10, 16, 22, 25, 26, 41, 42], which is in agreement with results obtained in the present paper. The applied medium salinity using both sodium chloride and calcium chloride caused a lowering of protein levels in tested plants leaves (Table 2).

Table 2

Changes of protein content [% d.m.] in leaves of root celery during salt treatments

Days	Salinity level of NaCl			Salinity level of $CaCl_2$		
	Background	100 mM	300 mM	Background	50 mM	150 mM
14	22.98	19.59	16.58	22.98	19.64	16.55
28	21.12	19.01	17.69	21.12	19.44	16.68
42	21.22	21.04	20.26	21.22	21.44	19.23
Average value	21.77	19.88	18.17	21.77	20.17	17.49
$LSD_{0.05}$	For time periods – 0.83 For salinity levels – 0.83			For time periods – 0.81 For salinity levels – 0.81		

The changes observed were proportional to the extent of soil salinity (Fig. 1). Taking into account average values of protein levels of all analysis periods, for higher levels of salinity (300 mM NaCl and 150 mM $CaCl_2$) decreases of protein content of 17 and 20 %, respectively, in celery leaves have been noticed as compared with background.

It is commonly known that medium salinity influences crop quality [11, 12, 32, 43–45]. Vegetable nutrition includes not only protein content, but really important are also bioorganic factors such as carbohydrates and vitamins.

The results obtained for soluble sugar contents in leaves of root celery have shown that applied salinity levels of soil using both natrium chloride and calcium chloride significantly have decreased contents of these compounds. During long-term exposure to salinity the sugar content was the lower the higher medium salinity was (Fig. 1). Taking average values, sugar levels for all analysis periods at lower levels of salinity (100 mM NaCl and 50 mM $CaCl_2$) decrease by 21 and 18 %, respectively, in celery leaves as compared to background plants (Table 3). For higher medium salinity levels of $CaCl_2$ (150 mM) and NaCl (300 mM) lowering of sugar content was much more significant (40 and 42 %, respectively).

Table 3

Changes of soluble sugar contents [% d.m.] in leaves of root celery during salt treatments

Days	Salinity level of NaCl			Salinity level of $CaCl_2$		
	Background	100 mM	300 mM	Background	50 mM	150 mM
14	3.78	3.13	2.40	3.78	2.98	2.25
28	3.28	2.86	1.54	3.28	2.82	1.87
42	3.48	2.30	2.16	3.48	2.83	2.17
Average value	3.50	2.76	2.03	3.50	2.88	2.12
$LSD_{0.05}$	For time periods – 0.13 For salinity levels – 0.13			For time periods – 0.16 For salinity levels – 0.16		

A large numbers of publications are available focusing on changes in sugar metabolism of plants under medium salinity [4, 5, 16, 22, 23, 32, 44, 46]. However, no general rule can be derived from the data presented there. In one case sugar level decreased during increasing doses of salinity, on the other hand, it was also found these, the higher the salinity level the higher the sugar content. From the present investigations the results presented coincide with those obtained by Sultana et al [22] in which a decrease of soluble sugars under salinity has been noticed.

According to our results, soil salinity to a large extent, also influenced vitamin C levels of root celery leaves (Table 4). In case of this component varied results were observed. To a large extent it was dependent on salt type and soil salinity level (Fig. 1). For average values of ascorbic acid contents of all analysis periods for lower levels of salinity of both NaCl and $CaCl_2$ a 10 % increase as compared with background has been noticed. For higher levels of salinity changes were variable. Soil salinity stress due to NaCl caused an almost 10 % decrease of vitamin C whilst stress induced by $CaCl_2$ caused a 7 % increase. These results coincide with some earlier ones. Sairam and Srivastava [18] observed a decrease whilst Keles and Öncel [47] and Eraslan et al [7] found an increase of the content of ascorbic acid under salt stress. Plants tolerance of salinity may induce changes in ascorbic acid levels. Vaidyanathan et al [6] have shown an increase of vitamin C in tolerant species independently of medium NaCl salinity level. In case of susceptible species increases of that component for lower levels of soil salinity have been observed.

Table 4

Changes of ascorbic acid content [mg^{-1} f.m.] in leaves of root celery during salt treatments

Days	Salinity level of NaCl			Salinity level of $CaCl_2$		
	Background	100 mM	300 mM	Background	50 mM	150 mM
14	33.14	39.90	29.33	33.14	39.24	36.19
28	40.66	43.22	37.33	40.66	46.65	43.59
42	50.18	53.48	45.42	50.18	51.28	53.12
Average value	41.33	45.53	37.36	41.33	45.72	44.30
$LSD_{0.05}$	For time periods – 3.51 For salinity levels – 3.51			For time periods – 2.79 For salinity levels – 2.79		

Conclusions

Medium salinity caused by sodium chloride and calcium chloride induced changes of total chlorophyll, protein, soluble sugars and ascorbic acid levels in leaves of root celery.

Changes of contents of the components determined were to a large extent dependent on soil salinity level.

Salt stress caused by both NaCl and $CaCl_2$ induced decreases of chlorophyll, protein and sugar contents in the tested vegetable. The stronger the soil salinity was the greater the decrease was.

Changes of levels of ascorbic acid in celery leaves were variable. For lower level salinity $CaCl_2$ and NaCl increases of that compound has been noticed.

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REAKCJA SELERA KORZENIOWEGO NA ZASOLENIE NaCl i $CaCl_2$

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Abstrakt: Przeprowadzone badania miały na celu określenie wpływu zróżnicowanego zasolenia podłoża NaCl i $CaCl_2$ na zawartość chlorofilu, białka, cukrów i kwasu askorbinowego w liściach selera korzeniowego (*Apium graveolens* L. var. *rapaceum.* (Mill.) DC). W eksperymencie wazonowym rośliny potraktowano doglebowo roztworami chlorku sodu (100 i 300 mM NaCl) i chlorku wapnia (50 i 150 mM $CaCl_2$). Uzyskane wyniki pozwalają wnioskować, że zawartość analizowanych składników w liściach selera była uzależniona od poziomu zasolenia podłoża oraz od soli użytej do zasalania. Zasolenie podłoża zarówno NaCl, jak i $CaCl_2$ prowadziło do obniżenia zawartości chlorofilu całkowitego, białka i cukrów rozpuszczalnych w liściach selera, przy czym odnotowane spadki zawartości tych składników były tym większe, im wyższy był poziom zasolenia gleby. Zasolenie gleby w dużym stopniu wpłynęło także na poziom kwasu askorbinowego w liściach selera. Zasolenie gleby $CaCl_2$, przy obu dawkach, powodowało wzrost poziomu kwasu askorbinowego. Inna reakcja wystąpiła pod wpływem NaCl, przy niższym poziomie zasolenia obserwowano wzrost zawartości witaminy C, podczas gdy przy wyższym poziomie zasolenia gleby odnotowano spadek zawartości tej witaminy.

Słowa kluczowe: zasolenie, seler, chlorofil, białko, cukry, kwas askorbinowy