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L-proline amount in callus tissues of Lancaster maize inbred lines under chloride load

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Salinity of soil and soil waters are actual problems of land utilization. Chloride salinity is the most common kind, in Ukraine it is dominated by sulphate and carbonate forms. Chloride salinity has a super-negative effect on the maize plant. Growth inhibition is observed already at 0.1% salt contents in soil, but the salt level of 0.3-0.4% provokes the wilting and plant death. Numerous metabolic cell abnormalities occur under the salt influence: inhibition of enzyme activities, photosynthesis, protein synthesis, disorders of respiratory processes [Dolgyh Ju. I., dis. phD: 384, 2005].

Protective plant response to the negative effect of abiotic factors is being induced by a lot of cell systems. One of the responses to the stress factors (salinity, drought and low temperatures) is the accumulation of free L-proline in the cells. The precursor of proline synthesis is glutamate or ornithine. Under the stress proline content increases due to the regulation of two opposite processes – the intensification of its biosynthesis and the inhibition of its catabolism.

Proline is an organic compound of low molecular weight which lightly resolves in water and forms colloidal polymer structures. Free proline and proline in the protein molecules are the required components of any plant cell. This

aminoacid is a component of the antioxidant protective system, it stabilizes the subcellular structures and macromolecules, regulates redox potential, participates in the modification of functions of mitochondria. Proline is a part of the signal transmission systems those control gene expression in response to stress [Anjum S. A. et al., Afr. J. Agric. Res. 6 (9): 2026-2032, 2011].

Ions Na^+ and Cl^- from the nutrient medium overcome the cell wall and enter the cell through anionic and cationic channels, penetrate through protein hydrate coverage and affect the noncovalent bonds that maintain the structure of the protein molecules. Proline does not penetrate through hydrate coverage and does not enter into direct contact with the proteins, but creates obstacles for the hydrate coverage destruction and the protein denaturation by ions [Alyohina N. D. et al., Physiology of plant: 636, 2006].

Proline is an important cell osmoprotector. On the one hand it protects proteins from denaturation and forwards their native conformation, interacting with them during stress. On the other hand, it helps to achieve the osmotic balance of cytosol with vacuoles and other cell organelles.

The subject of our work includes the determination of proline amount in maize callus tissues under chloride load and after its removal, the characterization of influence of sodium chloride on the regeneration potential of callus tissues.

Research material was represented by 5 inbreds of maize commercially valuable Lancaster germplasm (DK633/266, DK633/325, DK236, DK3070, DK6080) and 1 inbred of Polish germplasm (PLS61). Primary explants for induction of callus tissues were immature embryos, 1.5 mm in length. Callus tissue was initiated within 30 days on N6 medium (Chu Ch.-Ch. et al., Sci. Sinica 18: 659 – 668, 1975) modified with 690 mg/l L-proline, 100 mg/l inositol, 100 mg/l casein hydrolyzate, 1 mg/l 2,4-D, 0.1 mg/l abscisic acid and two levels of sucrose - 30 g/l or 60 g/l. Chloride load in vitro was simulated by adding into the medium for subcultivation sodium chloride in concentrations of 6, 30 and 60 g/l. The content of L-proline was determined for 330-day stabilized maize callus tissues obtained in two different ways. In the first version the callus tissue was subcultivated on the

N6 medium with 0 (control), 6, 30 or 60 g/l sodium chloride for 300 days right after the induction period. In the second version the callus tissue was subcultivated on the N6 medium with 0 (control), 6, 30 and 60 g/l sodium chloride during 210 days right after the induction period and maintained during the following 90 days on hormone free regeneration medium MS (Murashige T. A. et al., Physiol. Plant 15: 473 – 497, 1962) without sodium chloride.

Determination of the proline amount was performed by a modified method [Bates L. S. et al., Plant soil 39: 205-207, 1963]. Callus tissue sample (approximately 1 g) was poured by boiling distilled water (10 ml) and placed for 10 minutes in a boiling water bath. 2 ml of glacial acetic acid and 2 ml of ninhidryn reagent was placed into the clean test tube. Then 2 ml of extract was flowed to the same test tube. Samples were incubated for 1 hour in a boiling water bath and then rapidly cooled in ice. 10 ml of toluene for extraction of the proline was added to the obtained samples. The mixture was separated in a separatoring funnel. The color intensity of proline fraction was measured spectrophotometrically at the wavelength of 520 nm. Proline amount was calculated according to calibration curve constructed with crystalline proline. For the determination of proline amount the average sample from pieces of 3-5 typical calli was composed. Proline content was evaluated in 1 g of callus tissue. Data processing was carried out according to [Atramentova N. O. et al., Statistic methods in biology: 288, 2007]. Data in tables are presented as $\bar{x} \pm mt_{0.05}$, where $\bar{x}$ – the average value of the index, m – the error of average value, $t_{0,05}$ – Student criterion for significance level of 0.05.

Proline content in the maize callus tissues under the sodium chloride in the medium for subcultivation was raised compared to control and was being increased simultaneously with the increasing of sodium chloride concentration (Table 1). Proline content in control depended on genotype and fluctuated between 483.16 - 1,509.23 mg proline/g callus tissue. In general for induction medium applying 30 g/l sucrose the level of proline in callus tissues was not lower, but sometimes higher than for 60 g/l sucrose as without sodium chloride, so under its effect.

Table 1

Content of L-proline in maize calllus tissues under chloride load

Sucrose content in the medium for callus induction, *g/l*	Sodium chloride content in the medium for callus subcultivation, *g/l*	L-proline content in callus tissues, *mg L-proline/g callus tissue*
DK633/266		
60	0	483,16 ± 17,13
60	6	764,98 ± 18,45
60	30	1790,87 ± 19,88
60	60	2633,71 ± 19,24
DK633/325		
60	0	1431,33 ± 41,62
60	6	2573,36 ± 39,40
60	30	2698,25 ± 44,30
60	60	3621,32 ± 43,63
DK236		
30	0	1509,23 ± 22,20
30	30	2100,29 ± 16,40
30	60	4059,92 ± 27,69

Ninety days after the removal of chloride load the divergences of proline content for the callus tissues having been cultivated on different levels of sodium chloride have decreased (Table 2). Tested for inbred DK3070 levels of proline after removal of chloride load in callus tissues obtained on callusogenic medium with 30 g/l sucrose were higher then with 60 g/l sucrose. Regenerants were recovered only for inbred PLS61 on the medium for subcultivation with 6 g/l sodium chloride. Proline amount in callus tissues of PLS61 after the removal of chloride load was rather high compared to nonregenerable inbred DK3070.

Table 2

Content of L-proline in maize callus tissues after removal of chloride load

Sucrose content in the medium for callus induction, *g/l*	Sodium chloride content in the medium for callus subcultivation, *g/l*	L-proline content in callus tissues, *mg L-proline/g callus tissue*
DK3070		
30	0	250,18 ± 14,97
30	6	319,72 ± 18,17
30	30	390,46 ± 34,21
30	60	627,00 ± 44,58
60	0	192,57 ± 13,69
60	6	272,75 ± 23,90
60	30	259,11 ± 12,39
60	60	334,94 ± 38,22

DK6080		
30	30	1833,14 ± 54,71
30	60	5529,57 ± 53,06
PLS61		
60	6	2082,63 ± 21,25
60	30	2257,69 ± 46,52
60	60	2304,97 ± 40,18

The experimental data allow concluding that the response of maize callus tissues to chloride load leads to the accumulation of proline. The content of proline in callus tissues depended on the concentration of sodium chloride in the nutrient medium and increases with its magnification. Concentrations of sodium chloride in the nutrient medium of 30 and 60 g/l completely suppress regenerative potential of maize callus tissues, while 6 g/l sodium chloride permits the plant regeneration of certain genotypes.