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Callusogenesis in maize inbred DK212 under sodium chloride

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The resistance to salinity in maize is the important agricultural characteristics. Sodium chloride is one of the basic components of saline soils. The application of *in vitro* tissue culture method for creation of genotypes resistant to chloride salinity is a perspective direction of biotechnology investigations (Urechean, Bulg. J. Plant Physiol., Special Issue: 336-352, 2003). Regenerated plants resistant to sodium chloride have been obtained in barley (Ignatova, 2011), rice (Priya et al., Afr. J. Biotechnol. 10(36): 6947-6953, 2011), beet (Chugunkova, Physiol. and Biochemistry of Cultivated plants 6(242): 509-515, 2009), wheat (Koutoua et al., Int. J. Biosci. 1(4):12-25, 2011), potato (Homayoun et al., American-Eurasian J. Agric.&Environ. Sci. 11(5):729-732, 2011).

The increase of contents of sodium and calcium ions, the decrease of the concentration of potassium ions and the significant decrease of ratio K^+/Na^+ take place in tissues under salinity. Higher activity of superoxidedismutase was discovered in salt resistant forms and the decrease of enzyme activity under abiotic stress were revealed both in seedlings and callus tissues (Terletskaia, Biology of Plant Cells in Vitro and Biotechnology: 390, 2008). The salinity of the environment breaks osmotic and ionic homeostasis of cells and provokes the formation of a secondary oxidative stress. It is connected with the accumulation of active forms of oxygen whose oxidation effect is directed to lipids and leads to the disruption of structure and functions of membranes [Gurr et al., 2002]. Tissue culture method allows to simulate the salinity stress through adding sodium chloride to nutrient medium. Such a medium can be considered a selective one for screening of resistant maize cells and tissues to obtain resistant regenerants.

We investigated the callusogenesis under salinity in maize inbred DK212 which belonged to subplasm Oh43 of Lancaster germplasm. Immature zygotic embryos, 1-1.5 mm in length, were harvested on the 11th day after self-pollination from field donor plants and cultivated scutellum up on modified inductive N_6 medium (medium *Ind*) in the darkness. Calli derived in 60 days in culture were transplanted to next modified MS media: control medium (*C*), control medium + 0.1 Mol/l sodium chloride (*6C*) and control medium + 0.5 Mol/l sodium chloride (*30C*) and cultivated at the light. Medium *Ind* as compared to media *C*, *6C* and *30C* had

contained less sucrose for delaying the osmotic load because the osmotic pressure was created later with sodium chloride.

50.00 and 20.45 percent of green calli were observed respectively on media *C* and *6C* at the 30th day of cultivation at the light. Green coloration was disappearing through 30 days after its appearance.

Visually changes in calli sizes depending on sodium chloride contents were noted only to the 60th day of cultivation at the light. For estimation of callus cultivation specific diameters and specific raw weights of calli were measured (fig.1, 2). Specific dry weights and humidity of calli were determined at the end cultivation (table).

Values of specific raw weights and diameters of calli after 60 days of cultivation at the light were appreciably differed from analogical values fixed before transplantation. Maximal value of specific raw weight of calli was observed on the 120th day of cultivation at the light in all variants of the experiment. The specific diameter values achieved to the 150th day were preserved to the 210th day of cultivation at the light. The tendency of the decrease of specific dry callus weight and humidity under the increase of sodium chloride concentration was observed. Such a tendency indicates the inhibitory effect of sodium chloride on the accumulation of dry weight of calli (table). The reduction of calli humidity related to salt contents in the medium characterizes sodium chloride as an osmotic agent.

Thus, inhibitory effect of sodium chloride on callus growth depends on the age of culture and the sodium chloride concentration. 0.5 M sodium chloride is non-lethal concentration for tissue culture of maize inbred DK212 and can be further examined as a selective one.

Table

Maize callus characteristics on the 210th day of in vitro culture under different concentrations of NaCl

Concentration of NaCl in the medium, g/l	Number of calli cultivated	Specific raw callus weight, mg	Humidity of calli, %
0	44	7.42 ± 0.98	95,01 ± 6,64
6	44	6.07 ± 1.06	93,96 ± 7,26
30	45	2.60 ± 0.45	91,65 ± 8,34

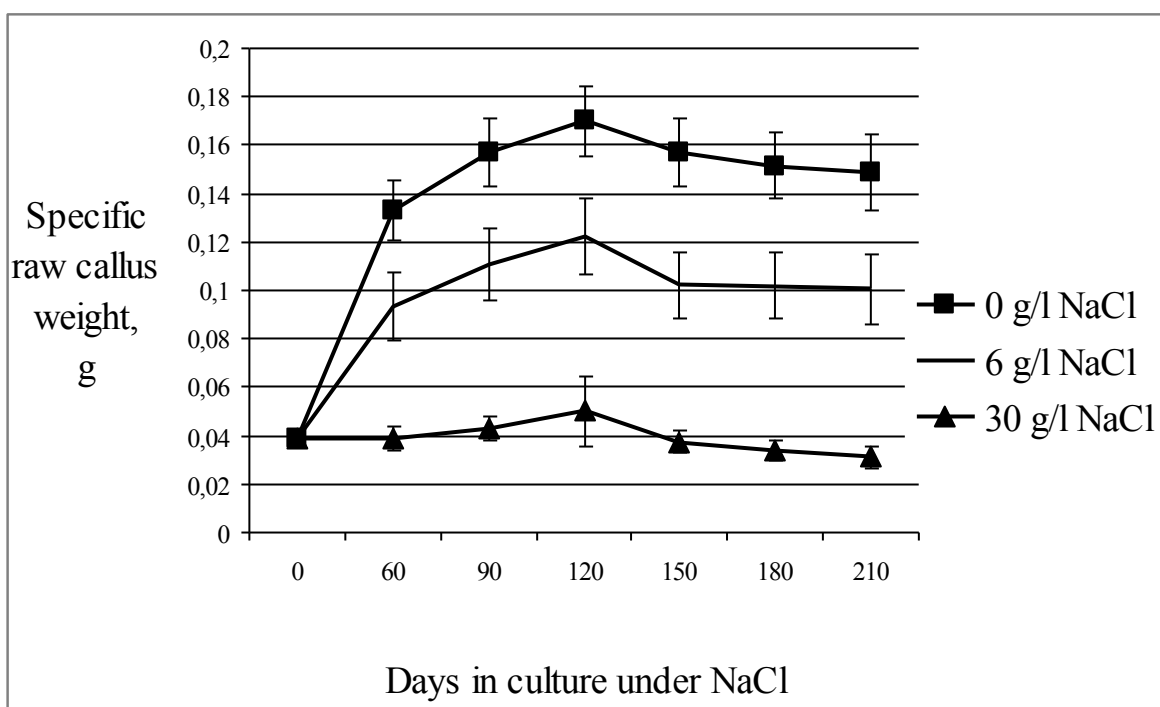


Fig. 1. The dynamics of specific raw callus weight under the influence of NaCl.

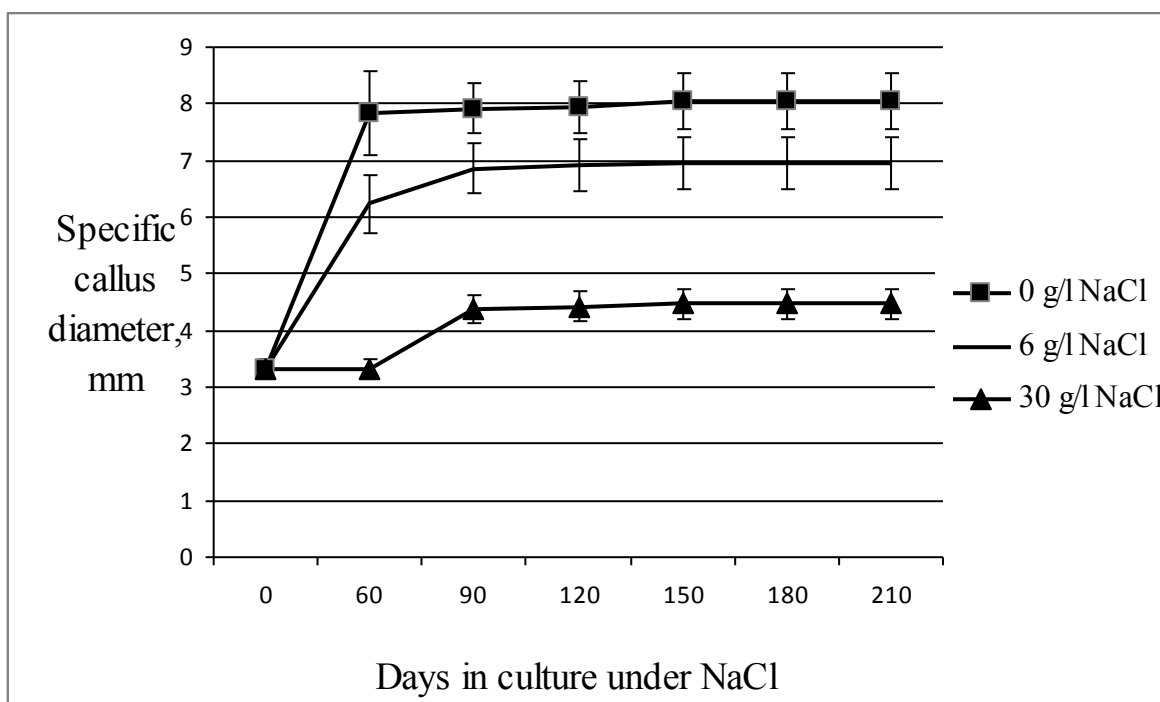


Fig. 2. The dynamics of specific callus diameter under the influence of NaCl.