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OSMOSIS OF *NICOTIANA TABACUM* L. CELL LINES UNDER SOAKING CONDITIONS

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Modern global ecology places categorical demands on biological sciences to produce plant forms with complex resistance to abiotic stresses. There is a need to develop new ideologies and improve methods for their in vitro production. Only such an integrated approach can ensure a major breakthrough in solving this scientific problem. Considerable attention is paid here to the latest biotechnological approaches that are being actively developed and implemented. along with undeniable achievements, there are problematic issues that require special attention. The range of such issues includes theoretical (biological), methodological and humanitarian problems.

There is now no doubt that stress tolerance is a polygenic characteristic. It combines the action of both cellular-level mechanisms and the reaction of the whole plant. The peculiarity is that the mechanisms implemented at the cellular level have priority.

Among the abiotic stresses, osmotic stress, or salinity, has the greatest harmful effect To reduce the harmful effects of salinity on plant yields, there is a need for salt-tolerant plant genotypes [1].

One of the most promising ways to solve the problem of obtaining stress-resistant plant forms is cellular selection. The selection of comprehensively resistant genotypes, then during each individual passaging, always involved changing the cultivation conditions. The modelling stress agent is a substance characterised by high toxicity in relatively small amounts. Therefore, it can cause significant cell damage. Cellular selection is becoming the main method of obtaining plant forms with unique characteristics. Heavy metal ions (HMI) are known to have such characteristics [4].

Ba^{2+} ion was used to create an in vitro model system. The selective concentration of barium ion was determined in previous experiments. In cell selection, the phenomenon of a stable cell culture is of great importance. A culture is considered to be resistant if it can only withstand stress pressure when growth processes are completely inhibited and resume proliferation only under normal conditions.

In our experiments, we selected cultures that maintained growth and development throughout the entire period of cultivation. To determine the stability, the relative biomass growth (Δm) was constantly monitored. Ba-RCL were obtained as a result of primary selection on medium with a lethal concentration of Ba^{2+} ion. After cell biomass growth on the selective selection medium, the callus was simultaneously passaged on control medium (normal conditions) and on selective media with Ba^{2+} .

The concentration of Ba^{2+} cations used was lethal for wild-type cell cultures.

Among the resistant tobacco cell lines, even ultra-resistant variants were isolated, which demonstrated proliferation in the presence of 5 mM barium cations, i.e., withstood a dose of toxicant 2.5 times higher than the lethal one.

It is known that Ba^{2+} ions affect the transport of K^{+} ions. Ionic interaction/antagonism has also been observed for other cations [2]. Thus, salinity resistance may be related to the kinetics of toxic cation transport or the structure of the transporters themselves. We also assume that the cross-resistance to Ba^{2+} ions as a salinity factor is associated with changes in membrane viscosity caused by changes in the degree of lipid saturation.

In our case, the cross-resistance of Ba-RCL is confirmed by the proliferation and growth of the culture on any selective medium. Most

likely, this event is due to the compartmentalisation of toxic ions. This assumption was confirmed by determining the free proline content in resistant cell lines.

Proline plays a crucial role in cellular mechanisms both as a component of proteins and as a free amino acid. Due to its cyclic structure, proline has limited configurational flexibility, which ultimately determines the stabilisation/destabilisation of the secondary structure of the protein. It is known that an increase in the extracellular protein pool is often associated with phosphorylation at tyrosine sites and determines the status of the culture [3, 5].

The use of Ba^{2+} ions in cell breeding has shown great potential for their suitability for obtaining plant forms with an increased level of salt tolerance. In fact, instead of a typical glycophyte, such as tobacco, a new genotype has emerged that corresponds to classical halophytes in terms of resistance (developing in the presence of 2.5% salinity).

Ba-CLCs respond to specific stress ions. The presence of Ba^{2+} cations affects the ionic balance by retaining K^+ , which, however, is not the only reason for salt resistance. The level of free proline in the RCL changes depending on the type of osmotic stress. In this case, proline plays a detoxifying role.

The involvement of heavy metal ions in cell selection has made it possible to obtain cell lines and plants resistant to salt stress. Such a promising biotechnological method makes it possible to obtain new stress-resistant genotypes of any crops.

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