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PRODUCTION AND STUDY OF CD RESISTANT TOBACCO PLANTS UNDER WATER STRESS CONDITIONS

ОТРИМАННЯ ТА ДОСЛІДЖЕННЯ CD СТІЙКИХ РОСЛИН ТЮТЮНУ ЗА УМОВ ВОДНОГО СТРЕСУ

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Global climate change and the acute shortage of fresh water, even for drinking, are hindering crop production. Among the abiotic stresses, osmotic stresses, such as salinity and water deficit, have the greatest harmful effect. Often, they act together, which further reduces the possibility of organism survival. There is a need to obtain plant forms that combine resistance to various stresses. Currently, new biotechnological methods are being actively developed that use micro and even nanotechnology [4, p. 1]. At the same time, the ideology of environmental safety is becoming a priority, both for the approach itself and for the product it produces. One of the most promising ways to solve the problem of obtaining stress-resistant forms of plants is considered to be cellular selection. However, like any technology, this method requires constant improvement [5, p. 1–2; 7, p. 1–3]. In vitro biotechnological methods are now beginning to outpace traditional methods of obtaining genetically modified plant forms in terms of their popularity.

The object of the study was tobacco plants of the Samsun and Dubec varieties. The purpose of the study was to determine the growth and physiological characteristics of cell cultures.

The cultures were initiated and grown on Hamburg's B_5 agarified medium until the study. Resistant cellular variants were obtained as a result of cell selection using "plating" of the optimized suspension cell culture into selective conditions. Resistant cells formed microcolonies. The frequency of colony isolation did not exceed 10^{-6}, which corresponds to the frequency of genetically altered forms. The resulting colonies were transferred to fresh selective medium, where they continued passivation for 2–3 passages to increase callus biomass; the duration of a single passage was 30–35 days. The vital activity was assessed by determining the relative increase in fresh biomass **(Δm): Δm = (mk – mi)/mi**, where **mi** is the mass of the culture at the beginning of the passage; **mk** is the mass of the culture at the end of the passage. A cell line is considered to be the progeny of a single genetically modified cell. The selective medium was created by adding lethal concentrations of the heavy metal cadmium (Cd^{2+}) ions to wild-type wheat cell cultures.

It is known that the water balance in plants can be maintained by a special class of proteins called dehydrins. This class includes LEA (late embryogenesis abundant proteins), proteins of the late stage of embryogenesis. A number of publications have noted that Cd^{2+} ions have a negative effect on LEA [3, p. 1–20; 6, p. 2–6]. Therefore, Cd^{2+} was used to obtain tobacco cultures resistant to water stress.

Earlier, the idea of using heavy metal ions Cd^{2+} in cellular breeding to produce plant forms resistant to water deficit was put forward and put into practice. The idea was based on the nature of the effect of Cd^{2+} ions on the water status of plants. Cd^{2+} ions significantly inhibit the activity of LEA, a group of dehydrin proteins. These proteins are directly related to the maintenance of the plant's water balance by moving water inside the body and transporting it between individual tissues [2, p. 74–76; 8, p. 11378–11379; 10, p. 255–269].

Therefore, according to our assumption, cell lines that are resistant to a lethal dose of Cd^{2+} will have an increased level of resistance to the modelled stress.

In general, a linear relationship between the water potential of the medium and the osmotic potential of cells in the stationary growth stage was established. It is noted that cells adapted to severe water stress do not keep their volume stationary, but change their turgor in proportion to the water potential of the medium.

In this way, active osmoregulation is carried out to prevent large-scale dehydration. The process is carried out by increasing the level

of endogenous components that reduce the water potential and provide a gradient that is optimal for water penetration.

This can be explained by the amount of mannitol in the culture medium. The presence of this compound significantly reduced the water potential of the medium. In this case, proline acts as an osmotic active substance that supports the hydration sphere of proteins.

At the same time, the presence of cadmium ions in selective media (lethal dose) can cause toxic damage at significantly lower doses. Therefore, in our opinion, the proline present in the control cultures was the result of the degradation of proline-containing cell wall proteins.

Proline-rich proteins (PRPs) are involved in the formation of cell walls. In their molecules, proline residues are organised into repeating motifs to which hydroxyproline is added. In stable cell cultures, the accumulation of free proline occurred due to synthesis [1, p. 1–13; 9, p. 1–17]. Under normal conditions, the level of free proline is maintained by its synthesis/degradation systems. However, under stressful conditions, only the synthesis system is active.

Plant resistance to osmotic stress is a polygenic characteristic. To achieve success, it is necessary to evaluate the maximum number of vital parameters available. This will create an opportunity to actively influence metabolism.

The latest biotechnology can be a priority in such experiments.

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