CELL SELECTION AS A MODERN BIOTECHNOLOGICAL DIRECTION FOR OBTAINING TOBACCO VARIANTS RESISTANT TO UNFAVOURABLE CLIMATE CONDITIONS

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Abstract. *On 26 November, the United Nations Environment Programme (UNEP) published its annual report on emissions into the Earth's atmosphere, prepared by 57 leading representatives of 33 research institutes from 25 countries. The authors of the report predict a 1.5-20% increase in global temperature by 2100. A 20 per cent rise in global temperature will bring hunger, poverty and floods to millions of people around the world. Recipes for preventing the effects of global warming have long been well known: improving energy efficiency and switching to renewable energy. At the same time, it is also pointed out that the global cost of combating warming will amount to USD 1.6-3.8 trillion annually. Therefore, the development and dissemination of various biological approaches to obtaining stress-resistant plants is a priority and relevant. This is especially true for cereals, including wheat. The importance of wheat as a human food and valuable animal feed is undeniable.*

The production of genetically modified plant forms follows a well-established algorithm and is therefore a biological technology.

Key word*. HMI, tobacco, cell selection, in vitro, cell culture, regenerant plant.*

Stress adaptation of agricultural plants has been and is a constant topic of fundamental and applied biology. The interest in this problem is clear. On the one hand, it is aimed at understanding the plant/environment (G/E) interaction; on the other hand, given the constant need of the growing population, efforts are being made to develop plants capable of withstanding abiotic stresses. The main goal of plant breeding is to obtain genotypes that combine high yields with real productivity stability over years and territories. In addition, plant products should be of high nutritional quality for food and feed and/or have other commercial value.

However, excessive or even moderate abiotic stresses are causing a decline in plant productivity around the world. This is also facilitated by anthropogenic pollution, which increases and modifies the stress load of natural factors by adding organic and inorganic xenobiotics. Nature faces the problem of complex pollution.

Although it is known that various environmental stressors never act separately, experimental studies of plant responses to abiotic stresses are usually limited to the study of changes that occur under the pressure of a single factor. This implies that stress always causes a complex of response reactions that contribute to the overall pathological process. Biotic and abiotic stress inducers have some related common signals and response pathways, and therefore plants can probably modulate the response to a specific factor through cross-interaction [7, 8].

Cell selection is an adequate method for obtaining resistant cell lines. The method of cellular selection has been used to obtain a significant number of plant forms with altered characteristics: auxotrophs, superproducers of certain amino acids, forms with an increased level of resistance to adverse environmental factors [5]. In all of these cases, direct selection was carried out using a single stress-forming agent. In cell selection, a single chemical compound is usually used to create a modelled stress [5, 9]. In the vast majority of experiments, the concentration of such a stressor is moderate, so the cellular variants obtained as a result of selection are qualified as "adaptants." Usually, regenerants from such adapted cells (if they are obtained) are not characterised by an increased level of stress resistance. Therefore, it is understandable that some researchers are cautious about the prospects of using cellular selection to obtain plants with an increased level of stress resistance [5, 8]. It is obvious that the traditional ideology of cellular selection does not meet the complexity of the problem.

It is known that abiotic stresses cause a complex of interrelated reactions that can occur simultaneously or alternately. Therefore, for the primary selection, it would be advisable to choose an agent that has both specific and general damaging effects. If a substance characterised by high toxicity in relatively small quantities and therefore causing significant cellular damage is chosen as a modelling stress agent, cellular selection can become the main method of obtaining plant forms with unique characteristics. Such characteristics are associated with heavy metal ions (HMIs), especially the group of HMIs that are toxic in residual amounts and are considered physiologically unnecessary. Such HMICs include: Ba^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, VO^{3-}, WO_4^{2-}. Despite their exceptional toxicity, these IRMs are characterised by different levels of study. While the ions Cd^{2+}, Hg^{2+}, Pb^{2+} are widely and comprehensively studied, the Wa2+ cation is relatively little known. As for anions, which in general are much more toxic than cations, they are the subject of research only in some cases [5, 13, 14, 15, 16].

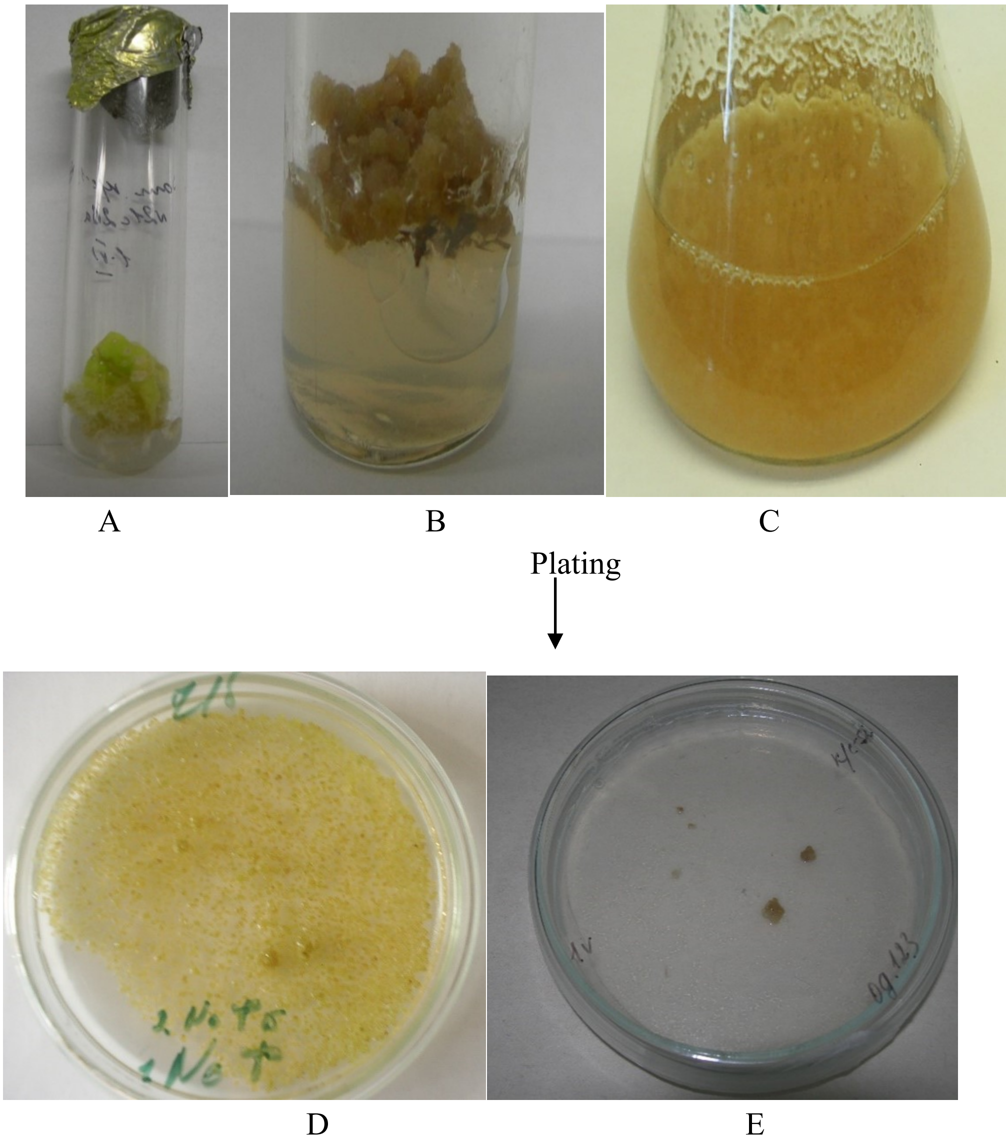
We proposed and practically tested the hypothesis (using tobacco as a traditional model object) about the possibility of cellular selection from HMI to obtain lines with cross-resistance to simulated osmotic stresses.

The following scheme was developed for this purpose: Cell selection with HMI (lethal concentration) → HMI-resistant cell line → cross-resistant cell line.

The following heavy metal ions were used to create the in vitro model system: Ba^{2+}, Cd2+. The selective concentration for each ion was determined in previous experiments. It was considered to be the smallest amount of stressor that stopped the development of the wild-type cell culture. If after returning to normal conditions, the vital activity of the culture was restored, the concentration of the stressor was increased. This prevented the selection of adaptants. If the ion used required special modifications of the medium, such changes were made taking into account the preservation of the qualitative and quantitative composition of the culture media. [1, 6].

It was necessary to establish the suitability of this approach in the selection of agriculturally important crops. The test was carried out using soybean cell cultures of a number of genotypes. In accordance with the developed scheme and rules of cellular selection, each stage of the experiment was accompanied by constant monitoring. Since the aim of the experiment was to select comprehensively resistant variants, the cultivation conditions were always changed during each passaging. The alternation of stress1 - control - stress1 or stress1 - control - stress2 was arbitrary. The number of passages on a single medium (cultivation period) was also arbitrary.

In cell selection (for further results), the phenomenon of a stable cell culture is of great importance. A culture that can only withstand stressful pressure with complete inhibition of growth processes and resumes proliferation only under normal conditions is often considered resistant. In our experiments, we selected cultures that maintained growth and development throughout the entire period of cultivation. To determine the resistance, the relative biomass growth rate (Δm) was constantly monitored [2, 3, 5, 10].



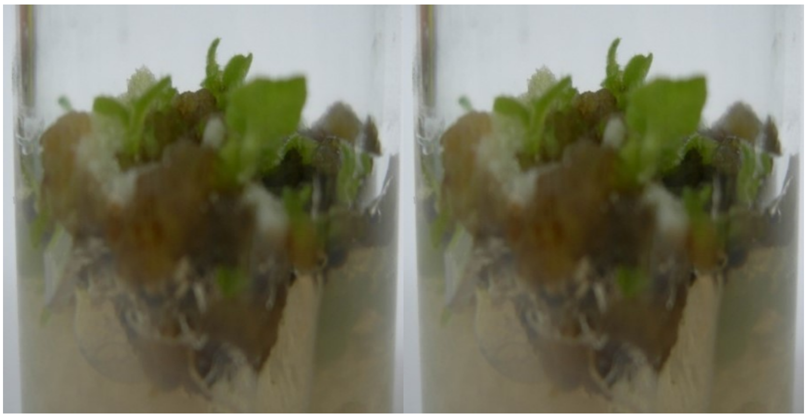
Cell selection, development of model selection systems, production of primary clones and stable cell lines.

A. Induction of primary callus; **B**. Growth of callus mass; **C**. Cell suspension culture; **D**. Cell growth under normal conditions; **E**. Selection of resistant clones on selective medium at a frequency of 10^{-6}.

A wide range of resistant plant cell lines was obtained on selective media containing lethal concentrations of Cd^{2+} and Ba^{2+} ions. During cultivation, the selected variants not only did not lose their level of resistance, on the contrary, in some cases it was possible to isolate clones with an increased level of resistance. Ba- and Cd-resistant cell lines also possessed complex resistance to various osmotic stresses.

During the whole period of the study, the cell cultures selected on selective media with HMI did not lose their characteristics. They were also preserved in the case of cultivation in the absence of a selective agent (in one of the experiment variants, cultivation on the control medium was 24 passages). "Cellular" resistance was also observed in cell cultures initiated from regenerant plants (secondary callus) that had never been subjected to stress.

In the course of experiments, the system: resistant cell line-resistant plant was investigated. The results of the study showed that, despite the existing differences, common resistance mechanisms are realised in the system at different levels (cellular, intact plant-regenerant).



A B

Direct organogenesis (**a**) and isolated regenerants on rooting medium (**b**)

The cell lines and plants derived from them retain and express the phenotypic traits for which the original cell selection was carried out in media with heavy metal ions.

The study of HMI resistant cell lines has shown that cell culture in general and cell selection as such has not exhausted its potential: as a method of studying the fundamental mechanisms of cell function under normal and stress conditions. It is a way to produce resistant plants. Cell selection, as a research ideology, is aimed at promoting environmental safety in experimental and production activities [1, 5, 9, 11, 17].

Cell selection using lethal doses of HMI may become one of the priority ways to obtain plants with unique characteristics. As a biotechnological method, cell selection has been sufficiently tested. It is difficult to surpass it in the study of fundamental mechanisms of cell operation in norm and under stresses of various types. Since the negative impact is carried out during the whole cell cycle, an opportunity to separate stress and adaptation reactions is created. This becomes extremely relevant for the identification of new determinants of resistance. In this case, it is not only a matter of guaranteed defence against stress lesions (synthesis of protector compounds), but also the work of enzymes of a new type. Thus, a new level of biological research is formed and a systematic approach to selection/evaluation of altered cell variants is created.

The new strategy proposed by us turned out to be an effective way to obtain cell lines and plants resistant to various abiotic stresses and toxic factors.

Modern biotechnologies are a reliable way to obtain improved plants Only in this way science can save biodiversity and life in general on our planet.

CONCLUSIONS

Heavy metal ions have a complex effect on living organisms. Resistance to them should cause significant changes that must be genetically determined

When selecting resistant cellular variants, cells that are characterised by stable growth in the presence of a constant stress factor, it is necessary to investigate the cause of resistance. Obviously, cross-resistance is possible when genetic and epigenetic changes are combined. This is particularly relevant because epigenetic changes are caused by mechanisms that normally operate during cellular differentiation.

The study of cell lines resistant to HMI has shown that cell culture in general and cell selection in particular has not exhausted its potential. As a method of studying the fundamental mechanisms of cell functioning under normal and stress conditions, it is difficult to surpass. As a way to produce resistant plants, it is a promising biotechnological approach. As a research ideology, it is aimed at promoting environmental safety in experimental and production activities.

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