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OBTAINING AND RETESTING OF COMMON CHICORY REGENERANT PLANTS DURING CELL BREEDING FOR RESISTANCE TO Ba^{2+} IONS

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Introductions. One of the most harmful environmental factors are heavy metals. Even in small doses, they have a negative effect on living organisms. The entry of heavy metals into the environment and their impact on biological objects is determined by the interaction of natural (content of elements in the soil) and anthropogenic factors. Heavy metals disrupt mineral nutrition, suppress the intensity of photosynthesis and respiration and the activity of cell division, reduce the content of pigments in leaves, which leads to a decrease in the vitality and productivity of the organism or its death [1–3].

Plant growing on land contaminated with heavy metal ions requires the use of special organizational, economic and agrotechnical methods. The use of new high-yielding varieties and hybrids adapted to growing conditions is one of the most effective, cheapest and environmentally friendly ways to increase the yield of agricultural crops under stress conditions [4]. For common chicory, a valuable agricultural crop of versatile purpose, this direction of research is particularly relevant. The lack of high-yielding, resistant to adverse environmental factors varieties is the main reason that restrains the expansion of areas under chicory [5].

The use of biotechnological methods saves experimental area, creates conditions for working with biological objects regardless of weather conditions, and

reduces time spent on selecting plant forms with desired characteristics. This contributes to increasing the efficiency of the breeding process. The cell culture provides the possibility of full control of the physical and trophic parameters of growing plant material and modeling the effect of a stress agent on the body, which is difficult to achieve when working with intact plants [6].

However, certain negative moments arise when selection is carried out at the cellular level: the difficulty of obtaining regenerating plants due to the loss of the tissue's ability to morphogenesis; resistance to stress at the cellular level is not always preserved at the level of the whole plant. This phenomenon arises as a result of the so-called «physiological habituation» or «cross-feeding» of cells [6].

Aim. The aim of our research was to obtain cell lines and regenerating plants of common chicory resistant to heavy metal ions. Barium chloride was chosen as a selective agent.

Materials and methods. The work used modified Schenck-Hildebrandt media, to which different concentrations (0.25; 0.5; 1.0; 1.5 mM) of barium chloride were added. During the staged cell selection, it was possible to select cell lines with maximum resistance to the stress factor (the limiting concentration of $BaCl_2$ in the nutrient medium was 1.0 mM). In order to obtain regenerating plants, the selected cell lines were transferred to regenerative nutrient media according to the Murasige_Skoog recipe, which were modified with increased concentrations of cytokinins (1.0-1.2 mg/l 6-BAP). Regeneration was carried out both in the presence of a stress agent and without it (control variant).

After microclonal reproduction, the obtained chicory plant material was transferred to selective growing conditions with the maximum permissible concentration of the stress factor for retesting.

The biomaterial was cultivated at a light intensity of 4 kL, a 16-hour photoperiod, a temperature regime of 20–24 ºC, and a relative humidity of 75 %.

Results and discussion. Callus tissue of common chicory has high rates of regeneration. When optimal conditions were created, morphogenesis occurred in 88.8% of callus tissues (the intensity of morphogenesis was 35–42 regenerants from

one microcallus weighing 50–60 mg). The process of morphogenesis took place both by organogenesis and by somatic embryoidogenesis [7].

Barium chloride exerts a strong toxic stress on the callus tissue of common chicory. During long-term cultivation of biomaterial under selective conditions, a decrease in growth indicators and inhibition of the morphogenic characteristics of callus tissues was observed. The regenerative capacity of the selected microcalli was preserved during three passages. Selected cell lines of common chicory resistant to stress factors were characterized by individual indicators of morphogenesis both on media with stress factors and in the control variant.

The most intense morphogenesis took place in the first passage on nutrient media without a selective factor. Depending on the genotype, the intensity of morphogenesis ranged from 3.67 to 7.35 plants from one microcallus. With each subsequent subcultivation, the regeneration rates decreased by 45.7–73.7 %

The intensity of morphogenesis of barium-resistant cell lines during three passages on average by genotype was 2.51 regenerants from one microcallus. The presence of barium chloride in regeneration media reduced the production of regenerating plants by 49.2 % compared to the control variant.

As a result of cell selection from barium-resistant callus lines, a total of 181 plants were regenerated, of which 33.7 % of plants were obtained on selective media in the presence of barium ions. To determine the stability of the obtained materials at the level of a whole plant, regenerating plants were cultivated on media with a selective factor in the maximum permissible concentration.

However, not all plant materials obtained from callus lines resistant to the stress factor retained signs of resistance. Survival of plant material was 71.8 % on average. Plant lines were obtained on control regeneration media, depending on the genotype of the plant, this indicator is at the level of 59.4–72.2 %. When retesting the plant material that was regenerated in the presence of a selective factor, this indicator was higher by 11.6 %, and on average by genotype it was 80.2 %.

The duration of cultivation of biomaterial in the presence of barium chloride in the nutrient medium had the greatest effect on the preservation of the sign of

resistance at the level of a whole plant. Regenerants obtained after the third cultivation provided almost one hundred percent survival when they were re-grown on environments with a stress factor.

Conclusions. Therefore, barium chloride creates a high stress pressure on the callus tissue of common chicory. When regeneration cell lines was carried out in the presence of a selective factor, a decrease in morphogenic indicators was observed in comparison with the control variant by 38.9–56.2 %. Retesting of plant lines obtained in the presence of a selective factor ensure the survival of the material at the level of 78.6–81.2 %, which is 15.5 % higher than the lines obtained on control regeneration nutrient environment.

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