SECTION 2. EXPERIMENTAL BOTANY

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EFFECT OF WATER STRESS ON TOBACCO VARIANTS OBTAINED UNDER *IN VITRO* CONDITIONS

ДІЯ ВОДНОГО СТРЕСУ НА ВАРІАНТИ ТЮТЮНУ ОТРИМАННІ В УМОВАХ *IN VITRO*

Bronnikova L. I.
Postgraduate Student
Dnipro National University
Oles Honchar Dnipro National University
Dnipro, Ukraine;
Junior researcher
Institute of Plant Physiology and Genetics National Academy of Sciences of Ukraine
Kiyv, Ukraine

Броннікова Л. І.
аспірант
Дніпровський національний університет імені Олеся Гончара
м. Дніпро, Україна;
молодший науковий співробітник
Інститут фізіології рослин і генетики Національної академії наук України
м. Київ, Україна

Zaitseva I. O.
Doctor of Biological Sciences, Professor,
Professor at the Department of Physiology and Introduction Plants
Oles Honchar Dnipro National University
Dnipro, Ukraine

Зайцева І. О.
доктор біологічних наук, професор,
професор кафедри фізіології та інтродукції рослин
Дніпровський національний університет імені Олеся Гончара
м. Дніпро, Україна

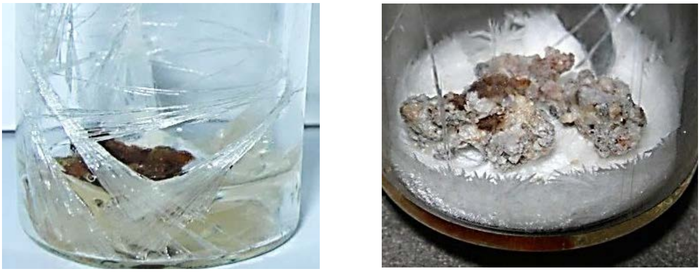
The problem of abiotic stresses is becoming a priority, given the increase in their spectrum and the expansion of the range of damage. However, drought has been and remains in the center of attention. Studies of water stress in vivo and in vitro have shown that dehydration is the primary cause of a significant limitation (cessation) of the body’s vital activity. The ability to control the movement of water, and with it the substances dissolved in it, inside/outside the cell is an active indicator of resistance. A high positive correlation between the ability to bind water and resistance has been reported in many plants [1]. *In vitro* biotechnological methods are now beginning to outpace traditional methods of producing genetically modified plant forms in terms of their popularity. However, like any method, they require constant improvement [8]. The use of new types of stressors will only enrich this proven method. Among the stress agents of a special type, heavy metal ions attract attention. Given the wide range of their harmful effects, their use in cell selection can create prerequisites for the isolation of unique cellular variants [8; 9].

The study of stresses caused by IPM goes hand in hand with the study of resilience. Resistance to HMI is considered a complex polygenic trait. We have obtained in vitro cell cultures resistant to cadmium ions. We hypothesized that it is possible to use lethal doses of heavy metal ions (HMI) to obtain forms with complex resistance. To confirm the objectivity of the hypothesis, cadmium ions were used in the experiments. Cadmium ions affect the water status of plants. This ion was chosen to obtain variants resistant to water stress [4; 5; 6; 7].

Cell cultures of tobacco varieties Dubek and Samsun were chosen as the subject of the study. For cultivation, a system of culture media was used that provided a solution to the task: selection of plant cell lines that are comprehensively resistant to various stress factors.

Cadmium ions were chosen to produce cell lines resistant to water stress. It is known that the water status of a plant is maintained by a number of proteins, including dehydrins. It has been noted that cadmium ions exert stress pressure on certain types of dehydrins LEA (late embryogenesis abundant proteins), proteins of the late stage of embryogenesis [2; 7]. Earlier, the idea of using heavy metal ions Cd^{2+} in cellular breeding to obtain plant forms resistant to water deficit was put forward and put into practice. The idea is 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, one of the groups of dehydrin proteins. These proteins are directly related to maintaining the plant’s water balance by moving water inside the body and transporting it between individual tissues. LEAs include dehydrins, proteins enriched with glycine, histidine, and lysine. It was found that the protective role of dehydrins is to prevent coagulation of molecules and maintain the integrity of cell membranes. This fact becomes especially relevant under dehydration, which served as a basis for our further actions [2; 8; 9].

Therefore, according to our assumption, cell lines resistant to a lethal dose of Cd^{2+} will have an increased level of resistance to the modeled stress. In general, a linear relationship between the water potential of the medium and the osmotic potential of cells in the stage of stationary growth has been established. It is noted that cells adapted to severe water stress do not keep their volume stationary, but change turgor in proportion to the water potential of the medium [4; 6; 7; 8; 9].



A **B**

Fig. 1. Cd2+-resistant tobacco cells cultured on selective medium with cadmium ions (a – passage ≥ 45 days; b – passage ≥ 60 days)

In this way, active osmoregulation is carried out to prevent large-scale dehydration. The process takes place 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.

Therefore, it was predetermined that cadmium-stable cotyledon cell lines of mulberry plants can maintain water status under conditions of severe water stress. Water stress was created by adding lethal doses of manitou. It was created by adding mannitol (non-metabolizable osmotic) or sea water salts. Experimental cell lines turned out to be resistant to water deficit and salinity.

A **B**

Fig. 2. Cultivation of tobacco plants under water stress in vitro (A); plants after 5 days of dehydration (B)

The decisive stage of cell selection for increasing the level of stress tolerance is regeneration of plants from selected cell lines. Often the regenerant plants do not retain the desired quality. Plants were obtained from Cd^{2+}-resistant tobacco cultivars (Fig. 2).

Since such forms with complex stability have not been described in the literature before, it was necessary to evaluate some indicators directly related to osmotolerance. We considered proline to be the key such indicator. Proline, a pyrrolidine-2-carboxylic acid, is characterized by a number of properties that make this compound a nonspecific stress protector. The accumulation of proline, as a compatible osmolyte, under salinity and water deficit has been established. Therefore, it was considered expedient to evaluate the role of proline in maintaining the viability of cell lines of cultures with complex resistance, as well as plants obtained from them [3; 8]. 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 and is the subject of further research.

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